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Myogenic precursor cell impairment in dermatomyositis and other myositis

Laure Gallay

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Myogenic precursor cell impairment in dermatomyositis and other myositis

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Se libérer de tout désir stérile, laisser la rivalité en chemin, apprendre à consentir à l'exigence de la vraie vie, rester humble devant les mouvements de l'âme, comme un serviteur devant plus grand que soi.
Lao Tseu, Philosophies Taoïstes

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White ways

Parfois il y a des regards en arrière qui laissent un petit vague dans l'âme.
Toutes ces intersections que l'on a prises en laissant des chemins inexplorés,
toutes ces portes laissées fermées, sans que l'on sache vraiment ce qu'il y avait derrière.
Ce ne sont pas des regrets, mais la perception de la multiplicité des possibles,
qui aboutit humblement à notre unique chemin.
Parfois je me retourne, et je réalise que ces intersections croisées, ne s'effacent pas vraiment.
Comme une marque dans mon cœur, dans ma peau,
comme les vestiges d'une construction ancienne que le temps érode sans violence,
mais dont les soubassements demeurent.
Comme une mémoire des possibles.
Elle a de plus l'intelligence de ne garder que le beau et le bon, la bienveillance et la tendresse,
d'éteindre le feu des obstacles rencontrés, des inadéquations des interactions.

Part of us, deep inside.

On pourrait lutter, nager contre le courant.
Inutiles et vains combats.
On peut l'accompagner, avec parfois une souriante lucidité,
gardant libres les souvenirs du cœur.
Laisant les portes fermées contenir tous leurs rêves,
et parfois revenir la nuit sur la pointe des pieds, pour doucement regarder par la serrure.

Petits nous, petits nous.

Il faut une vie entière pour apprendre à vivre.
Il en faut la moitié pour prendre conscience de ces ombres qui nous suivent, les apprivoiser,
pour finalement comprendre...
Elles ne nous enlèvent aucun soleil.
Elles restent docilement collées à nos semelles engluées de rêve.

Le vent se lève...

RESUME / ABSTRACT

ABSTRACT

Idiopathic inflammatory myopathies (IIM) constitute a heterogeneous group of diseases defined by non-infectious nor toxic acquired myopathies. Amongst them, dermatomyositis (DM) is the most frequent IIM, occurring during child and adulthood, and is potentially life-threatening. Its diagnosis is based on the association of muscle weakness, inflammatory infiltrates on histological muscle sections and auto-antibodies in the serum. Etiopathogenesis, that has mainly focused on adaptative immunity, remains largely unknown. In DM, the necrosis/regeneration balance is detrimental to muscle integrity, with a chronic course and poor clinical outcome for some patients. Nevertheless, the capacity of muscle regeneration has been barely studied. In normal injured muscle, regeneration is achieved by myogenic precursor cells (MPCs) that undergo myogenesis process to build new myofibers. The aim of our project was to evaluate DM-derived MPC capacity to undertake myogenesis.

Methods.

After establishing specific selection criteria, I selected DM (severe [n=5] and mild [n=3]), and healthy controls (HC [n=5], sex and age paired) from the Hospices Civils de Lyon database and obtained corresponding samples from the hospital biobank. Supplemental investigation was performed on other IIM derived-MPCs: immune-mediated necrotizing myopathy (IMNM) (n=3), inclusion body myositis (IBM) (n=3), and anti-synthetase syndrome (ASS) (n=3). Myogenesis was explored *in vitro* using specific MPC culture for the evaluation of proliferation, differentiation and fusion. Proliferation was evaluated as the incorporation of a thymidine analog into DNA during proliferation. Terminal myogenic differentiation was evaluated by myogenin expression, an essential transcription factor of myogenic differentiation. Cell fusion was quantified as the number of nuclei incorporated into myotubes. Quantitative analysis was performed using Image J software, and statistical analysis was performed by mean comparison (t test or ANOVA). Similar methods were used to evaluate the impact of interferon blockers (JAK / STAT inhibitor) on myogenesis. Senescence was analyzed by flow cytometry with fluorogenic substrate of SA- β Galactosidase, a senescence-specific marker.

Results.

Analysis of MPC proliferation indicates that DM-derived MPCs exhibited a significantly lower proliferation than HC-derived MPCs ($p=0.0020$). Terminal myogenic differentiation was also strongly impaired in severe DM-derived MPCs while less altered in mild-DM-derived cells ($p=0.001$). MPC fusion was significantly altered in DM-derived MPCs with a dramatic decreased observed in severe DM and to a lesser degree in mild DM ($p<0.0001$ for each). These results were recapitulated for other IIM, with altered myogenesis identified for IMNM (n=3), IBM (n=3), but not for ASS (n=3). Through cellular and molecular investigations, we found increased senescence in DM derived-MPCs, and potential involvement of interferon type I in myogenesis impairment. Indeed, sDM and mDM derived-MPCs myogenesis was improved even restored when culture was performed with JAK/STAT inhibitor, a downstream interferon pathway blocker or IFN receptor blocking antibody.

Conclusion.

These results show that skeletal muscle regeneration is impaired in DM due to altered capacities of MPCs to proliferate, to differentiate and to fuse. This may explain in part the muscle alterations and muscle weakness that are observed in patients. These results support the hypothesis of intrinsic alterations of MPC properties in DM, potentially linked with interferon pathway, and participating to the pathogenesis of the disease.

Key words: Myositis, dermatomyositis, myogenesis, senescence, interferon

RÉSUMÉ

Les myopathies inflammatoires idiopathiques (MII) constituent un groupe hétérogène de maladies musculaires acquises. Parmi elles, la dermatomyosite (DM) est la plus fréquente, survenant à tous les âges de la vie, et pouvant engager le pronostic vital. Le diagnostic repose sur l'association d'une faiblesse musculaire, d'infiltrats inflammatoires sur la biopsie musculaire et d'auto-anticorps dans le sérum. La pathogenèse apparaît multifactorielle, avec de nombreuses études concentrées sur l'immunité adaptative, et reste à ce jour incomplètement comprise. Dans le muscle de DM, la régénération ne compense pas la nécrose, et ceci induit une évolution chronique. Néanmoins, la capacité de régénération musculaire a été très peu étudiée dans ces pathologies. Dans le muscle normal, la régénération du muscle lésé se fait par un processus de myogenèse initié par les cellules souches musculaires (CSM) et permet une réparation *ad integrum* du tissu lésé. L'objectif de ce projet est d'évaluer la capacité des CSM dérivés de DM à réaliser le processus de myogenèse.

Patients et méthodes :

Une sélection de patients DM (sévère [n = 5] et légère [n = 3]) et des contrôles sains (CT [n = 5]) (appariés sexe et âge) a été effectuée dans la base de données des Hospices Civils de Lyon dont les échantillons étaient disponibles en biobanque. Une étude complémentaire a été réalisée sur d'autres patients IIM : myopathie nécrosante auto-immune (MNAI) (n = 3), myosite à inclusions (IBM) (n = 3) et syndrome des anti-synthétases (SAS) (n = 3). La myogenèse a été explorée *in vitro* par la culture de CSM pour l'évaluation de leur prolifération, de leur différenciation en myocytes et la fusion des myocytes en myotubes. La prolifération a été évaluée par incorporation d'un analogue de la thymidine dans l'ADN. La différenciation myogénique terminale a été évaluée par l'expression cellulaire de la myogénine. La fusion cellulaire a été quantifiée comme le nombre de noyaux incorporés dans les myotubes. L'étude de la sénescence a été faite par cytométrie en flux avec un substrat fluorescent de SA- β Galactosidase, marqueur spécifique de la sénescence. Enfin, l'impact de l'inhibition de la signalisation interféron sur la myogenèse *in vitro* a été évalué avec un inhibiteur des kinases JAK et un anticorps bloquant.

Résultats :

La prolifération des CSM de DM est significativement plus faible que celle des CSM de CT ($p = 0,0020$). La différenciation myogénique terminale est également très altérée dans les CSM de DM sévères, et à moindre niveau dans les cellules de DM légères ($p = 0,001$). La fusion est significativement diminuée dans les DM avec une diminution majeure pour les DM sévères et à un degré moindre pour les CSM de DM légères ($p < 0,0001$ pour chacun). Ces résultats ont été reproduits pour les autres IIM, sauf les ASS. Grâce à des investigations cellulaires et moléculaires complémentaires, nous avons trouvé une sénescence accrue dans les CSM de DM *in vitro*, et une implication potentielle de l'interféron de type I dans l'altération de la myogenèse. En effet, la myogenèse des CSM de DM a été améliorée voire restaurée par l'adjonction des inhibiteurs de la signalisation interféron.

Conclusion :

Ces résultats montrent que la régénération du muscle squelettique est altérée dans la DM en raison de la capacité altérée des CSM à réaliser le processus de myogenèse. Cela peut expliquer en partie les altérations musculaires et la faiblesse musculaire observées chez les patients. Ces résultats soutiennent l'hypothèse d'altérations intrinsèques des propriétés des CSM dans la DM, potentiellement liées à la voie de l'interféron et participant à la pathogenèse de la maladie.

Mots clef : Myosite, dermatomyosite, myogenèse, sénescence, interféron

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From Gallay et al 2019.

ABBREVIATIONS

ADM: Amyopathic Dermatomyositis
APCs: antigen-presenting cells
APS1/APECED : Autoimmune polyglandular syndrome type 1 / autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
ASS: anti-synthetase syndrome
CD: cluster differentiation
CK: creatine kinase
COX: cytochrome c oxidase
DCs: dendritic cells
DM: dermatomyositis
DMD: Duchenne Muscular Dystrophy
ELISA : enzyme-linked immunosorbent assay
eNOS: endothelial NOS
ER: Endothelial reticulum
ERK: Extracellular signal-regulated kinases
FAP: Fibro/adipogenic progenitors
FBS: Fetal Bovine Serum
GAF: gamma-interferon activated factor
GAS: gamma-interferon activated sequence
GSK3b: Glycogen synthase kinase 3 beta
HGF: hepatocyte growth factor
HMGB1: High mobility group box 1 protein
HMGR: hydroxy-3-methyl-gulatrlyl-CoA reductase
HRK: harakiri
HSPGs: heparan sulfate proteoglycans
IBM: Inclusion Body Myositis
IFN-I: type-I interferon
IFN: Interferon
IFNAR: Interferon alpha Receptor
IIM: Idiopathic Inflammatory Myopathies
IKKb: nhibitor of nuclear factor kappa-B kinase subunit beta
IL: interleukine
ILD: interstitial lung disease
IMNM: Immuno mediated inflammatory myopathies
iNOS: inducible NOS
IRF: Interferon Regulatory Factors
IS: immunosuppressive drugs
ISFG3: interferon-stimulated gene factor 3
ISG15: Interferon-Stimulated Gene 15
ISGs: IFN-stimulated genes
JAK: Janus Kinase
jDM: juvenile dermatomyositis
MAC: Membranous Attack Complex = C5b9

MAPK: Mitogen Activated Protein Kinase
MCP-1 : monocyte chemoattractant protein 1
MDA5: Melanoma differentiation-associated gene 5
mDM: mild dermatomyositis
MHC-I: Major Histocompatibility Complex class I
MPCs: Myogenic Precursors Cells
MRFs: Myogenic Regulator Factors
MRI: Magnetic resonance imaging
MuSC: Muscle stem cells
MxA: Myxovirus Resistance A
Myf5: Myogenic factor 5
MyHC : myosin heavy chain
MyoD: myoblast determination protein 1
NF- κ B: Nuclear factor-kappa B
nNOS: neuronal NOS
NO: nitric oxide
P/S: Penicillin/Streptomycin
PAH: Pulmonary arterial hypertension
PBMC: peripheral blood mononuclear cell
pDCs: plasmacytoid Dendritic Cells
PI3K (Phosphoinositide 3-Kinase
PIC: Polyinosinic-polycytidylic acid [poly(I:C)]
PM: polymyositis
RIG-I: retinoic-acid inducible gene 1
RNA: Ribonucleic Acid
RNF5: ER-Bound RING Finger Protein 5
SDH: succinate dehydrogenase
sDM: severe dermatomyositis
SIMOA : Single molecule array
STAT: Signal transducer and activator of transcription
TIF-1 γ : transcription intermediary factor 1
TLRs: Toll-Like Receptors
TNF: tumor necrosis factor
VEGF: Vascular endothelial growth factor

PREAMBLE

As a result of my dual curriculum of Science and Medicine, my work of PHD consists in translational research. The work is articulated around idiopathic inflammatory myopathies, including clinical and basic science research. The results of my PHD will therefore be presented in two different parts, first presenting a work focused on an extremely rare disease (around 250 cases reported across the international literature) in order to further delineate clinically and histologically this peculiar entity. In the second part, the work will focus on the study of pathogenesis of idiopathic inflammatory myopathies, and more specifically on the regeneration of muscle during the muscular damage. To this end, through ex-vivo and in vitro works, myogenic precursor cells and their myogenesis properties have been evaluated. Some underlying pathway potentially involved in regeneration alteration have been evaluated, others are discussed in the perspectives.

INTRODUCTION

1. Idiopathic Inflammatory Myopathies (IIM)

1.1. IIM clinico-sero-pathological definition

IIM constitute a heterogeneous group of diseases defined by non-infectious nor toxic acquired myopathies. Described in 1975 by Peter and Bohan, IIM have been initially classified as dermatomyositis (DM) and polymyositis (PM) (Bohan and Peter, 1975). Then, further deciphering and understanding of IIM led to the identification of other novel clinic-sero-pathological entities: inclusion body myositis (IBM), anti-synthetase syndrome (ASS) and immune-mediated necrotizing myopathies (IMNM) (Allenbach et al., 2018; Griggs et al., 1995; Mammen et al., 2020). IIM represent rare diseases with global prevalence around 6/100,000. Other very rare subgroups have also been identified, such as immune checkpoint inhibitor related myositis, focal myositis (FM) and granulomatous myositis.

The main symptom is muscle weakness, with various muscular subgroup impairment, most frequently proximal and symmetrical. Clinical features depend from the IIM subgroup, varying from mild to life-threatening phenotypes, with various degrees of extramuscular impairment including skin, lung, or heart impairment (Table 1).

Muscular pathology analysis identifies different phenotypes, with inflammatory infiltrates within the muscle as the major criteria associated with various kinds of impairment depending on the diagnosis (Table 1). Necrosis and regenerating myofibers (stained by CD56 or NCAM), Major Histocompatibility Complex class I (MHC-I) sarcolemmal overexpression and membrane attack complex (C5b9) capillar or sarcolemmal abnormal staining are other pathological hallmarks that help to achieve histological IIM diagnosis.

The recent discovery of different myositis-specific auto-antibodies (Abs) permitted to revisit the old classifications. Dermatomyositis is associated with anti-Mi2, small ubiquitin-like modifier activating enzyme (SAE), nuclear matrix protein-2 (NXP2), TIF-1 γ or melanoma differentiation-associated gene 5 (MDA5) Abs. Immune-mediated necrotizing myopathies are associated with anti-signal recognition particle (SRP) or 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) Abs. One third of inclusion body myositis' patients also presented anti-cytosolic 5'-nucleotidase 1A (cN1A) Abs (Benveniste et al., 2016). This tool is now part of the current diagnosis process, and is a useful warning for the clinicians since some specific auto-antibodies have been identified as associated with underlying severe extra-muscular

impairment or para-neoplastic syndrome. For example, amyopathic DM associated with severe pulmonary and skin impairment have been associated with anti-MDA5 DM, cancer have been found significantly associated with anti-transcription intermediary factor 1 (TIF-1 γ) DM and calcinosis with anti-NXP2 DM (Fiorentino et al., 2013; Fodil et al., 2016). IIM outcomes depend on the clinical subtype, even if chronic course takes place for most patients. Current therapies include immunosuppressive drugs with first and foremost corticosteroids.

	DM	IBM	IMNM	ASS
Mean age (years)	40 and younger	50 and older	40 and younger	40 and younger
Muscle impairment	Mild to severe, proximal and symmetric (upper limbs++, deltoids)	Progressive, proximo-distal, asymmetric	Severe, proximal and symmetric (lower limbs ++, psoas)	Mild to severe, proximal and symmetric
Swallowing disorder	possible	frequent	possible	possible
Extra muscular involvement	Yes	No	Yes	Yes
Cutaneous	DM rash (Gottron's papules, heliotrope rash, shawl sign), skin ulcers, alopecia	No	No	Mechanic hands, Raynaud syndrome Phenomenon DM-like rash
Pulmonary	Interstitial lung disease (ILD) Pulmonary arterial hypertension (PAH)	No or aspiration pneumonia	Rare	ILD PAH
Heart	Rare	No	Possible	Rare
Cancer association	Particularly in anti-TIF-1 γ DM	No	Particularly in seronegative IMNM	Rare
other	Calcinosis, panniculitis, limb edema	Facial muscle impairment		Arthralgia and arthritis
CK level	Moderately increase	Normal or subnormal	Highly increase	variable depending of the auto-Ab
Auto-Ab	Anti-TIF-1 γ Anti-Mi2 Anti-NXP2 Anti-SAE1/2 Anti MDA5	Anti-Cn1A	Anti-HMGCR Anti-SRP	Anti-Jo1 Anti-PL7 Anti-PL12 Anti-EJ, OJ Anti-KS, HA, Zo
Treatment	Steroid, IS, IVIg	No	Steroid, IS, IVIg	Steroid, IS, IVIg
Outcome	Remission possible, relapse possible	Progressive course, no remission	Remission possible, relapse frequent	Remission possible, relapse possible

Table 1. IIM diversity regarding clinical, biological and outcomes phenotypes.

	DM	IBM	IMNM	ASS
Myofibers	Perifascicular atrophy, MxA overexpression	Rimmed vacuoles Invasion of non-necrotic fibers Mitochondrial abnormalities	Necrotic myofibers Regeneration	Perifascicular necrosis, nuclear actin aggregates
Vascular domain impairment	Yes, Capillary dropout, endothelial tubuloreticular inclusions	No	No	No
Inflammatory infiltrates	Yes, perifascicular and perivascular	Yes, endomysial and perimysial	No or rare	Yes
Composition	CD4	CD8	CD68	CD4
MHC-1 staining	Positive	Positive	Positive	Positive
C5b9 staining	Capillary deposition	No or rare	No or rare Sarcolemmal	Perifascicular sarcolemmal
Fibrosis/adiposis	Variable	Variable	Variable	Variable

Table 2. Simplified muscle biopsy features found in various IIM.

During the PHD, the IIM subgroups and classification has been reported through an educational chapter. (**Myopathies acquises idiopathiques, Diagnostic difficiles en médecine interne 2018, L Gallay, O Landon Cardinal and O Benveniste**). This task of synthesis has allowed the better understanding of these rare diseases.

Myopathies acquises

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Points clés

Les **myopathies acquises idiopathiques** correspondent à un groupe de pathologies du muscle se développant en dehors du cadre héréditaire et qui peuvent survenir à tous les âges de la vie. Ce groupe compte les **myopathies idiopathiques inflammatoires**, les **myopathies toxiques**, **iatrogènes**, **endocriniennes** et **infectieuses**. La prévalence faible, ainsi que la multiplicité des phénotypes rendent leurs diagnostics parfois complexes. Il s'agit de **maladie de système** au cours desquelles plusieurs organes peuvent être atteints. Dans l'évolution, des complications graves peuvent les accompagner, notamment des atteintes cardiaques et pulmonaires. Contrairement aux myopathies génétiques, un **traitement** est souvent disponible, soulignant ainsi la nécessité pour le clinicien de savoir identifier ces maladies, et d'en connaître les principaux **diagnostics différentiels**.

INTRODUCTION

La masse musculaire variant classiquement entre 28 et 35 % du poids du corps, le muscle est un organe primordial. Il

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est régulé par des mécanismes d'une infinie précision permettant son fonctionnement et son adaptation, dont la pathologie est complexe. En effet, une grande variabilité des atteintes musculaires est rencontrée au cours de la pratique médicale. Ce chapitre a pour vocation de détailler les éléments diagnostiques des pathologies musculaires acquises, comprenant les myopathies inflammatoires idiopathiques (MII), les myopathies toxiques, les myopathies iatrogènes, les myopathies endocriniennes et les myopathies infectieuses (Figure 1). Ces dernières présentent une importante hétérogénéité de phénotype et d'agent causal, et ne seront pas traitées dans ce chapitre. Par ailleurs, certaines maladies musculaires héréditaires, notamment les myopathies métaboliques et certaines dystrophies, peuvent parfois présenter un phénotype clinique similaire, et doivent être considérées comme des diagnostics différentiels à éliminer (Figure 1). Elles seront abordées à la fin de ce chapitre.

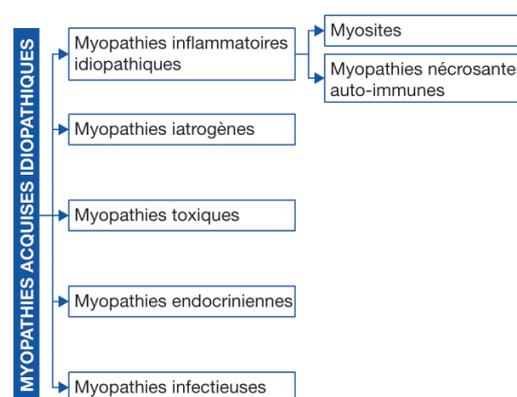


Figure 1 – Myopathies acquises idiopathiques.

I. MYOPATHIES IDIOPATHIQUES INFLAMMATOIRES (MII)

Les myopathies idiopathiques inflammatoires (MII), dont la première classification a été proposée en 1975 par Peter

et Bohan, regroupent l'ensemble des atteintes musculaires acquises, conséquences de phénomènes dysimmunitaires [1]. Ce sont des maladies rares, dont la prévalence, variant en fonction du sous-type, est proche de 6 pour 100 000. Leur cadre nosologique est en constante évolution, et ce grâce à la meilleure compréhension de leurs physiopathologies, à l'amélioration des différents outils qui servent à leurs diagnostics, tel que l'avènement des auto-anticorps spécifiques des myosites (ASM), ainsi qu'à la meilleure description de la sémiologie anatomopathologique du muscle. On identifie à l'heure actuelle quatre principaux sous-types de MII : les dermatomyosites (DM), les syndromes de chevauchement (OM pour *overlap myositis*, dont le syndrome des antisynthétases (SAS) et les myosites associées à des connectivites), les myosites à inclusions (IBM pour *Inclusion Body Myositis*), et les myopathies nécrosantes auto-immunes [2]. Plus rarement sont décrites d'autres entités, comme les myosites granulomateuses (MG), les myosites secondaires aux immunothérapies (MSI) et les myosites focales (MF) (Figure 2). La polymyosite, entité décrite initialement, est devenue rare, en raison du reclassement, avec les classifications et outils actuels, soit en myosite de chevauchement, soit en myosite à inclusions, en fonction de leur phénotype et de la présence d'auto-anticorps.

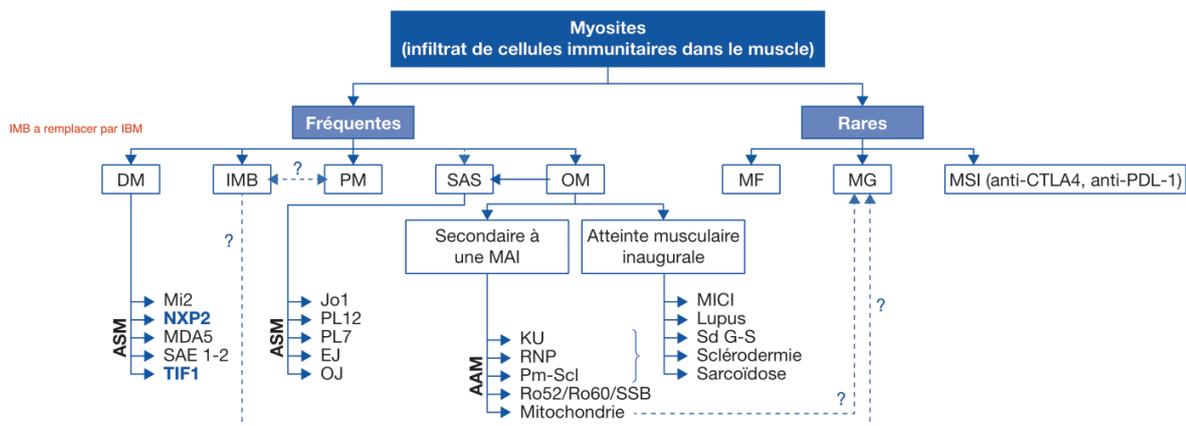
A. MYOSITES

L'ensemble du groupe myosite se définit par une atteinte du muscle avec infiltrats inflammatoires, ayant des conséquences cliniques et fonctionnelles plus ou moins sévères. Il regroupe des pathologies relativement fréquentes (DM, OM, SAS, IBM) et d'autres beaucoup plus rares, dont

seulement quelques dizaines de cas ont été publiées (MG, MSI, MF) (Figure 2). L'atteinte musculaire peut s'associer à d'autres atteintes, notamment articulaires, pulmonaires, ou cutanées. Les éléments clefs permettant de porter le diagnostic sont cliniques (critères démographiques, la durée et le type d'évolution, la topographie et le caractère symétrique ou non de l'atteinte musculaire, les symptômes extramusculaires incluant les manifestations pulmonaires, articulaires et les signes généraux, le terrain dysimmunitaire sous-jacent, une néoplasie associée, *etc.*), paracliniques (élévation des CPK, voir chapitre « Interprétation d'une élévation de la créatinine phosphokinase », vol. 2), lésions inflammatoires à l'IRM ayant ou non une sélectivité musculaire, aspect myogène à l'EMG, immunologiques (présence d'ASM ou de AAM (anticorps associé aux myosites ; voir chapitre « Conduite à tenir devant une recherche positive des anticorps anti-nucléaires », vol. 1), histologiques (topographie des lésions, variation de la composition des infiltrats, présence de nécrose, marquage sarcolemmal du CMH-I et capillaire du complexe d'attaque membranaire [C5b9]). Ce dernier outil diagnostique devra être réalisé autant que faire se peut avant l'initiation du traitement, et orienté sur un groupe musculaire dont l'atteinte a été objectivée en EMG ou IRM.

1. Évolution des concepts

Avec l'avènement de nouveaux outils diagnostiques et la meilleure compréhension de ceux déjà existant, les classifications des myosites se modifient régulièrement [2, 3]. Ainsi, l'entité polymyosite tend à disparaître, menant au reclassement de la majorité de ces cas vers les IBM, OM, DM sans atteinte cutanée, voire une myopathie héréditaire inflammatoire (myopathie fascioscapulohumérale,



ASM : auto-anticorps spécifique des myopathies idiopathiques inflammatoires (MII) ; AAM : auto-anticorps associé aux MII ; DM : dermatomyosite ; MF : myosite focale ; MG : myosite granulomateuse ; IBM : *Inclusion Body Myositis* ou myosite à inclusions ; MSI : myosite secondaire aux inhibiteurs de checkpoint ; MICI : maladie inflammatoire chronique de l'intestin ; OM : *Overlap Myositis* ou myosite de chevauchement ; PM : polymyosite ; SAS : syndrome des anti-synthétases ; Sd G-S : syndrome de Gougerot-Sjögren. Surlignage bleu clair : MII identifiées comme fréquemment associées aux cancers.

Figure 2 – Différents sous-groupes dans les myosites.

dysferlinopathie, etc.). Par ailleurs, dans le sous-groupe des myosites de chevauchement, le SAS apparaît clairement comme le chef de file de par la spécificité moléculaire de ses anticorps, son profil histologique particulier et à sa prévalence non négligeable. Ces myosites de chevauchement regroupent, les myosites phénotypiquement similaires au SAS, s'intégrant dans des tableaux de connectivites mixtes associées à des AAM (anti-RNP, anti-Ku, anti-Pm/Scl), et les myosites associées à des connectivites identifiées. L'évolution ultime attendue est que chaque myosite soit individualisée par l'ASM/AAM qui l'accompagne.

2. Myosites communes

a. Dermatomyosites

La dermatomyosite (DM) est l'entité la plus connue du groupe des myosites. Elle peut toucher tous les âges (forme pédiatrique et de l'adulte), et se définit par une atteinte cutanéomusculaire avec un profil anatomopathologique relativement spécifique.

D'un point de vue pathogénique, l'interféron de type I et le complément semblent jouer un rôle majeur. En effet, il a été montré une augmentation des transcrits interféron dans le muscle malade, ainsi que des accumulations de complexe d'attaque membranaire (C5b9) au niveau des petits vaisseaux. Ceci entraîne des souffrances tissulaires qui aboutissent d'une part au recrutement local de cellules inflammatoires, et d'autre part à une atrophie des tissus adjacents.

Cliniquement, le déficit musculaire peut apparaître avant, pendant, ou après l'apparition des symptômes cutanés (qui peuvent manquer). Il s'agit d'une atteinte proximale, généralement symétrique, dont l'intensité peut varier d'une atteinte infraclinique (DM cliniquement amyopathique), à une atteinte très sévère ayant d'importantes conséquences fonctionnelles. Une atteinte des muscles striés pharyngés et du haut œsophage peut se traduire par une dysphagie proximale et une odynophagie. Moins fréquente, l'atteinte des muscles paravertébraux peut engendrer un déficit axial pouvant aller jusqu'à la camptocormie. L'atteinte cutanée classique est composée d'une association d'un rash héliotrope (c'est-à-dire le plus souvent dans les zones photoexposées, de couleur violette à lilacée, parfois prurigineux ou brûlant), de papules de Gottron et d'un signe de la manucure (Figure 3). Elle peut dans certains cas différer, notamment avec des lésions ulcérées et nécrosantes, ou encore de la calcinose. Le rash héliotrope, avec ou sans œdème, est caractéristique dans sa localisation péri-orbitaire, et est aussi nommé « érythème lilacé des paupières » (Figure 3). Les papules de Gottron sont localisées en regard des petites articulations des mains, des coudes, des genoux ou des pieds. Le signe de la manucure correspond à un érythème péri-unguéal avec mégacapillaires, visibles macroscopiquement, ou à l'aide de la capillaroscopie. Il est assez caractéristique, sans pour autant être pathognomonique. Des localisations au cuir chevelu sont possibles. Il est important de souligner que les manifestations cutanées de la DM peuvent préexister à l'atteinte

musculaire et persister alors que la myopathie est en rémission. D'autres atteintes extramusculaires sont possibles, notamment pulmonaires (pneumopathie interstitielle ou syndrome restrictif dû à une atteinte diaphragmatique), articulaire (arthralgie, polyarthrite), ou encore cardiaque. La survenue d'une DM dans un contexte de néoplasie est décrite dans près de 30 % des cas, et sera discutée ci-après. Il est à noter que le pronostic est réservé dans les cas d'atteintes pulmonaires graves ou d'association à un cancer.



Figure 3 – Atteinte dermatologique typique lors de la dermatomyosite. A et B, deux exemples du rash héliotrope; A, de type œdème lilacé des paupières, B, signe du V, C, érythème en bande du dos des mains (ou érythème de Gottron) avec papules de Gottron (2°, 3° métacarpo-phalangiennes) et signe de la manucure (érythème péri-unguéal).

D'un point de vue paraclinique, les enzymes musculaires sont normales dans 20 à 30 % des cas. L'analyse anatomopathologique montre des lésions inflammatoires comprenant des infiltrats de lymphocytes T (CD4 et CD8), une atrophie périfasciculaire, et des dépôts endocapillaires du complexe d'attaque membranaire (C5b9).

Des variations notables du tableau clinique et de la gravité apparaissent au sein du groupe des DM. Grâce à la découverte d'ASM associés aux DM, des sous-groupes dont les caractéristiques anatomo-cliniques et évolutives sont plus homogènes ont été identifiés. Actuellement, cinq auto-anticorps ont été décrits dans les DM (anti-Mi2, NXP-2, SAE 1-2, TIF1γ, MDA-5), chacun reconnaissant une molécule cellulaire différente, et s'intègre dans un tableau particulier. Dans certains cas de DM, il n'y a, à ce jour, pas d'ASM identifié [4]. De manière intéressante, les cas pédiatriques semblent fréquemment associés à des tableaux de vasculopathie multi-organe, de fasciite et de calcinose, mais exceptionnellement à des atteintes pulmonaires ou des néoplasies [5].

DM associée à l'anticorps anti-Mi-2

Décrit en 1976 par Reichlin et al., l'anticorps anti-Mi2 est le premier anticorps à avoir été associé aux myosites [6]. Il reconnaît un complexe protéique impliqué dans la transcription de l'ADN [7]. Sa présence a été identifiée dans moins de 10 % des DM, et s'associe plutôt à une DM d'évolution subaiguë ayant une bonne réponse au traitement [8]. Au cours de cette association, les DM présentent une symptomatologie relativement typique, avec une atteinte dermatologique associant rash héliotrope et papules de Gottron. La faiblesse musculaire proximale peut être sévère. En

dehors de l'atteinte cutanée, il n'y a en général pas d'autre atteinte extramusculaire. Les CPK sont relativement élevées. L'analyse anatomopathologique montre un profil habituel de DM, avec un muscle très endommagé, notamment une anisodiamétrie marquée (variation de la taille des fibres musculaires au sein d'un même échantillon), d'importants stigmates de régénération (fibres CD56+), un marquage CMH-I périfasciculaire, et des dépôts de C5b9 plutôt sarcolemmal que capillaire. La question de l'association de l'anticorps anti-Mi2 et du cancer n'est pas encore tranchée [8, 9], néanmoins, dans l'analyse d'une cohorte de 48 patients séropositifs Mi2, Hengstman *et al.* posaient la question d'un sous-type d'anticorps anti-Mi2 reconnaissant la partie N terminale de la molécule et étant plus spécifiquement associée au cancer [8]. **Cets** résultats sont à confirmer.

DM associée à l'anticorps anti-SAE 1-2

Cet anticorps, décrit en 2012 par Betteridge *et al.*, reconnaît une enzyme impliquée dans les modifications post-traductionnelles des protéines, ciblant les sous unités SAE1 et SAE2. Sa fréquence varie entre 1,5 et 8% des DM [10-12]. Cliniquement, l'atteinte cutanée classique survient généralement avant le tableau musculaire, lequel semble plutôt modéré, voire absent. Pour autant, la survenue d'une dysphagie semble être fréquente. La présence de symptômes généraux comme la fièvre ou l'amaigrissement ont été rapportés [10]. Les atteintes extramusculaires, notamment pulmonaires ou articulaires semblent, quant à elles, peu fréquentes. D'un point de vue histologique, les éléments habituels sont retrouvés de manière modérée, avec un marquage des dépôts de complément très faible, voire absent.

Cet anticorps ne semble pas associé à la survenue d'un cancer, néanmoins, l'étude d'une cohorte japonaise de 7 patients publiée en 2015, relatait un taux de cancer à 57%, invitant à la vigilance, notamment chez les patients d'origine asiatique [11].

DM associée à l'anticorps anti-NXP2

L'anticorps anti-NXP2 cible une protéine de la matrice nucléaire qui régule la transcription et le métabolisme des ARN. Il a été découvert initialement chez des enfants atteints de DM associée à une calcinose [13, 14]. Cet anticorps représente 13 à 24% des ASM associés aux DM [15, 16]. Sa positivité est rapportée au cours de cas pédiatriques et adultes avec une prédominance masculine. Dans une étude de 56 cas, Albayda *et al.* rapportaient un phénotype alliant atteinte musculaire proximale usuelle, atteinte distale (membres supérieurs [35%] et inférieurs [25%]) et axiale (48%). Des myalgies et l'existence d'une dysphagie étaient fréquemment retrouvées (relativement 48 et 62%). Les atteintes cutanées étaient de forme inhabituelles : calcinose diffuse (30%) ou forme œdémateuse diffuse (36%), ce qui est soutenu par plusieurs travaux [17-20]. La survenue d'une telle atteinte cutanée semble précoce dans l'évolution de la DM à anti-NXP2. Lors de cette association, peu d'atteintes extramusculaires sont rapportées. Concernant le risque de pathologie néoplasique, 9% des patients de la cohorte sus-citée avaient un diagnostic de cancer associé à leurs DM. Une étude récente évoque l'association de cet

auto-anticorps aux cancers masculins [21]. Concernant les données histologiques, en plus de l'aspect classique de DM, on note un dépôt modéré de complément au niveau sarcolemmal, avec un très faible marquage des capillaires par le C5b9, associé cependant à une perte en capillaire pouvant être sévère.

DM associée à l'anticorps anti-MDA5

MDA5 (*Melanoma Differentiation Associated Gene 5*) est une hélicase cytoplasmique appartenant à la famille des récepteurs RIG (*Retinoid acide Inducible Gene*) like, qui est impliquée dans l'immunité innée antivirale. L'anticorps anti-MDA5 a été identifié en 2009 [22], et est associé à la DM dans des proportions variables (13 à 30% des cas) [23]. L'hypothèse d'un facteur génétique a été publiée, notamment avec l'antigène HLA-DRB1 qui semblerait pouvoir être un locus à risque pour cette association [24]. Sa positivité est corrélée à une atteinte particulière, parfois qualifiée de DM amyopathique. En effet, l'atteinte musculaire, très modérée, voire absente, est associée à des symptômes extramusculaires comprenant une vasculopathie périphérique, une pneumopathie interstitielle parfois foudroyante, et une atteinte articulaire pouvant mimer un rhumatisme inflammatoire. La vasculopathie périphérique se traduit par la survenue d'un acrosyndrome, de nécroses cutanées à type d'ulcère, ou encore de nécroses digitales (Figure 4) [25]. Les CPK peuvent être normales ou modérément élevées. D'un point de vue anatomopathologique, les DM associées à l'anticorps anti-MDA5 semblent présenter un aspect histologique particulier. Dans une étude de 16 patients, Allenbach *et al.* rapportaient les éléments suivants : le marquage sarcolemmal de CMH-I est très modéré ; le marquage CMH-II est négatif ; les infiltrats sont très peu nombreux, regroupés en clusters sur le pourtour des vaisseaux périnervaux. Il n'y a pas de marquage par le complexe d'attaque membranaire (C5b9) sur les capillaires et la perte en capillaire est moins importante que dans les DM classiques. D'autres anomalies peuvent être retrouvées, notamment les « tubules ondulants typiques » de la paroi endomysiale des capillaires, et la positivité de certaines fibres au marquage NOS2 et HSP70, molécules impliquées dans le stress cellulaire [26].



Figure 4 – Atteinte dermatologique au décours d'une DM à anticorps anti-MDA5 : nécrose digitale.

La prise en charge thérapeutique des DM associées à l'anticorps anti-MDA5 requiert une attention particulière. En effet, en cas d'atteinte pulmonaire interstitielle d'évolution rapide, la mortalité est élevée. Un traitement immunosuppresseur agressif et un management en unité de soins intensifs peuvent être nécessaires [5]. Matsushita *et al.* ont rapporté une corrélation entre le taux d'anticorps et la gravité de la maladie, pouvant aider le clinicien à déterminer l'intensité du traitement et le suivi des patients [27]. D'autres auteurs font une corrélation entre la survenue d'une atteinte pulmonaire grave lorsque la ferritine initiale est > 1 200 UI/L [28]. Cependant, en l'absence d'étude prospective de validation, on ne peut à ce jour recommander une telle attitude.

DM associée à l'anticorps anti-TIF1γ

TIF1γ (*Transcriptional intermediary factor-1-γ*) est un facteur de transcription nucléaire impliqué dans la voie de signalisation du TGFβ (*Tumor Growth Factor-β*), jouant un rôle majeur dans la différenciation cellulaire et la carcinogénèse. L'anticorps nucléaire anti-TIF1γ a été découvert par Targoff *et al.* en 2006 [29]. Il est retrouvé dans 13 à 20 % des myosites, avec une légère prédominance féminine. La présence d'un anti-TIF1γ chez l'adulte doit rendre particulièrement vigilant le clinicien. En effet, sa positivité a été retrouvée dans 84 % des cas de DM associées à un cancer se développant dans l'année précédant ou suivant le diagnostic [30]. Certains patients développent une néoplasie dans les 4 années qui précèdent ou suivent le diagnostic de DM.

Cliniquement, la présence de cet anticorps est associée à une faiblesse musculaire proximale touchant surtout les membres inférieurs. En cas d'association au cancer, les patients sont plus âgés (moyenne d'âge 60 ans), les lésions cutanées, parfois pseudo-psoriasiformes, semblent avoir une évolution souvent plus chronique, et la survenue d'une dysphagie apparaît assez fréquente [30]. Les atteintes pulmonaires et articulaires sont rares. Histologiquement, les éléments notables sont le marquage dense du C5b9 sur les capillaires et la présence de fibres contenant des vacuoles (différentes des vacuoles bordées de la myosite à inclusions). Quelques fibres en nécroses sont aussi observées, ainsi qu'un marquage intense du pourtour des fibres par le CMH-I et des infiltrats inflammatoires (lymphocytes T CD4/CD8 et macrophages) diffus [30]. Afin de dépister une éventuelle néoplasie, il est nécessaire de surveiller ces patients régulièrement par un examen clinique complet et un bilan paraclinique orienté, et ce pour les 4 à 5 ans suivant le diagnostic.

DM séronégatives

Dans un certain nombre de DM, dont l'expression clinique et anatomopathologie classique permet le diagnostic, aucun ASM n'est à ce jour identifié. Dans la majorité de ces cas, il n'y a pas d'éléments cliniques particuliers, et l'évolution est favorable sous traitement. Il est néanmoins important de souligner que dans les DM séronégatives, des cas de néoplasies synchrones ont été rapportés, notamment chez les patients de plus de 50 ans ayant des lésions cutanées nécrosantes.

b. Myosite de chevauchement

Groupe émergent de myosite, les OM sont des maladies touchant essentiellement l'adulte, dont la sévérité est variable. Les OM correspondent à un regroupement de plusieurs entités (Figure 2) :

- les **myosites associées à la présence d'un ASM du groupe des anti-ARNt-synthétases** (Anti-Jo1, PL7, PL12, EJ, OJ, KS, YRS, ZO, SC, JS), qui se traduisent cliniquement par une association plus ou moins marquée d'atteintes musculaire et extramusculaires (pulmonaires, articulaires, cutanées, *etc.*);
- les **myosites associées à la présence d'un AAM** (Anti-Ro52/60, SSB, Ku, U1RNP, PM-Scl) dont l'atteinte musculaire fait partie de symptômes s'intégrant dans un syndrome de chevauchement ;
- les **myosites observées au décours d'autres maladies auto-immunes systémiques** identifiées (telles que le syndrome de Gougerot-Sjögren (SGS), la sclérodermie, *etc.*).

Syndrome des anti-synthétases

Le SAS est une connectivite d'expression clinique variable, dont la physiopathologie reste relativement mal comprise. Une médiation humorale ainsi que la rupture de tolérance prenant place au niveau pulmonaire sont les pistes actuelles de compréhension de sa pathogénèse. Le SAS est défini par la présence d'auto-anticorps anti-ARNt-synthétase, associé à un phénotype clinique conjuguant plusieurs symptômes : myosite, pneumopathie interstitielle diffuse (80 %), arthralgies (77 %), syndrome de Raynaud (48 %), fièvre (31 %), et mains de mécanicien (27 %) (Figure 5) [31-33]. La pondération des différentes atteintes varie pour chaque cas, en fonction du sous-type d'anticorps présent, et durant l'évolution de la maladie. À ce jour, dix auto-anticorps ciblant les ARNt-synthétases, enzyme impliquée dans la traduction des ARN en protéines, ont été identifiés (anti-Jo1, PL7, PL12, EJ, OJ, KS, YRS, ZO, JS, SC) (Tableau 1) [16, 34-40]. Ils sont retrouvés dans environ 20 % des MII. La détection des 5 premiers se fait en routine grâce à des kits commerciaux d'immunoblotting. De manière intéressante, il a été rapporté une corrélation entre le titre de l'anticorps anti-Jo-1 et l'activité de la maladie, sans conséquence thérapeutique à ce jour [41].

Dans le SAS, l'atteinte pulmonaire est un facteur pronostic majeur. L'expression clinique varie d'un tableau a- ou pauci-symptomatique d'évolution lente, à une atteinte amenant le patient en réanimation en quelques jours ou semaines, cette dernière forme étant beaucoup plus rare. Elle précède parfois la myopathie, ou s'inscrit dans le cadre d'un tableau amyopathique [42, 43]. L'imagerie pulmonaire a donc une place importante dans la prise en charge de ces patients [44]. La composante pneumologique varie entre les sous-groupes, et est notamment reconnue comme plus fréquemment dans les SAS associés aux anticorps anti-PL7 et PL12 [45, 46]. Elle se manifeste le plus souvent par une atteinte parenchymateuse (pneumopathie interstitielle diffuse de plusieurs types : pneumopathies interstitielles non spécifiques, pneumopathie interstitielle commune), pneumopathies organisées et plus rarement vasculaire (hypertension pulmonaire précapillaire ; voir chapitre « Pneumopathies interstitielles



Figure 5 – Atteinte dermatologique au décours d'un syndrome des anti-synthétases. Mains de mécanicien au cours d'un syndrome des antisynthétases (lésions hyperkératosiques, fissuraires, douloureuses des doigts).

diffuses », vol. 2). Une hypertension pulmonaire (HTP) pré-capillaire est présente dans moins d'un cas sur dix, mais cette atteinte est sévère et associée à une moindre survie [45]. Ceci souligne, d'une part, la nécessité de rechercher les anticorps anti-ARNt-synthétases dans des tableaux parfois incomplets et, d'autre part, le caractère indispensable de la réalisation d'un bilan cardiopulmonaire exhaustif (tomodensitométrie thoracique, épreuves fonctionnelles

respiratoires, échographie cardiaque, cathétérisme droit en cas d'échographie cardiaque pathologique) au diagnostic, et au cours du suivi des patients. Du fait de sa potentielle gravité, l'atteinte pulmonaire conditionne le pronostic de la maladie. Elle requiert souvent une prise en charge collaborative et la mise en place d'un traitement rapide, parfois intensif combinant corticothérapie, immunosuppresseur et mesures pneumologiques (arrêt du tabac, évaluations régulières).

L'analyse anatomopathologique du muscle tend à identifier un profil particulier, relativement homogène quel que soit le sous-groupe de SAS. Il associe des infiltrats de cellules inflammatoires préférentiellement périnysiaux (lymphocytes T, B, plasmocytes, macrophages), ainsi qu'une myonécrose des fibres et une augmentation du marquage sarcolemmal de CMH-I et II dans la région périfasciculaire. Des dépôts de complément (C5b9) sont présents dans le sarcolemme des fibres musculaires adjacentes au périnysium. En microscopie électronique, des agrégats de filaments d'actine dans les noyaux des fibres musculaires ont été rapportés dans 70 % des cas, avec une bonne spécificité [47]. L'inflammation musculaire peut être absente, ceci malgré un profil clinique et immunologique compatible.

Chevauchement avec présence d'auto-anticorps associés aux myosites

L'atteinte musculaire peut être soit la porte d'entrée dans une myosite de chevauchement, soit un nouveau symptôme intervenant dans un tableau de connectivites en chevauchement. L'association d'une atteinte musculaire à un AAM est parfois associée à un phénotype particulier, comme décrit ci-dessous.

ASM	Fluorescence	Type histologique	Fréquence	Âge de diagnostic
Anti-Mi-2	Cytoplasmique	DM	10 % des DM	Adulte
Anti-MDA-5	Cytoplasmique	DM, DMCA	13-30 % des DM	Enfant et adulte
Anti-SAE1/2	Nucléaire	DM	1,5-8 % des DM	Adulte
Anti-NXP-2	Nucléaire	DM	13-20 % des DM	Enfant et adulte
Anti-TIF1 γ	Nucléaire	DM	13-20 % des DM	Enfant et adulte
Anti-Jo-1	Cytoplasmique	SAS	11-21 % des MII	Enfant et adulte
Anti-PL-7	Cytoplasmique	SAS	2-5 % des MII	Adulte
Anti-PL-12	Cytoplasmique	SAS	2-3 % des MII	Adulte
Anti-OJ	Cytoplasmique	SAS	< 1 % des MII	Adulte
Anti-EJ	Cytoplasmique	SAS	< 1 % des MII	Adulte
Anti-KS	Cytoplasmique	SAS	< 1 % des MII	Adulte
Anti-YRS	Cytoplasmique	SAS	< 1 % des MII	Adulte
Anti-ZO	Cytoplasmique	SAS	< 1 % des MII	Adulte
Anti-JS	Cytoplasmique	SAS	< 1 % des MII	Adulte
Anti-SC	Cytoplasmique	SAS	< 1 % des MII	Adulte
Anti-HMGCR	Cytoplasmique	MNAI	10 % des MNAI	Enfant et adulte
Anti-SRP	Cytoplasmique	MNAI	15 % des MNAI	Enfant et adulte

Tableau 1 – Fluorescence, fréquence des différents ASM (anticorps spécifiques des myopathies idiopathiques inflammatoires [MII]).

L'anticorps anti-Ku, anticorps à fluorescence nucléaire, a été identifié avec des prévalences rapportées jusqu'à 23 %. Il est associé à un phénotype pathologique proche du syndrome des antisynthétases, comprenant : myosite (myalgies, faiblesse musculaire proximale, dysphagie, CPK élevées), arthrite, syndrome de Raynaud, pneumopathie interstitielle diffuse (semblant être plus fréquente chez les patients ayant des symptômes musculaires) [31]. L'anticorps peut être isolé ou associé à des maladies auto-immunes, comme la sclérodémie systémique ou le lupus érythémateux systémique [48]. En dehors des atteintes pulmonaires qui semblent assez corticorésistantes, l'évolution, notamment musculaire, est le plus souvent favorable.

L'anticorps anti-mitochondrie. Une étude de 2012 rapportait une prévalence de cet auto-anticorps chez 11,3 % des patients au sein d'une cohorte de 212 myosites [49, 50]. Cette étude soulignait la fréquence des atteintes cardiaques (58 %), et l'identification dans 25 % des biopsies musculaires analysées de granulomes. L'anticorps anti-mitochondrie peut être retrouvé isolément ou en association avec une cirrhose biliaire primitive [49, 50].

L'anticorps Anti-Pm/Scl, dont la fluorescence est nucléaire, peut être associé à la sclérodémie systémique mais peut aussi être retrouvé dans un syndrome de chevauchement avec atteinte musculaire sans sclérodémie. Sa positivité en cas de scléromyosite a été évaluée à environ 10 % des cas. Les patients associant sclérodémie, atteinte musculaire et anti-PM/Scl sont jeunes. Sur le plan clinique, l'atteinte cutanée globale est modérée en dehors de la survenue plus fréquente de calcinose cutanée, l'atteinte pulmonaire est majoritairement fibrosante, alors que l'HTP est plus rare [19].

L'anticorps anti-U1RNP est un auto-anticorps à fluorescence nucléaire qui peut être retrouvé positif au décours d'une myosite (10-15 %) [31, 51], essentiellement dans le cadre d'une connectivité mixte associant myosite, pneumopathie interstitielle et polyarthrite. Cette atteinte semble associée à une maladie d'assez bon pronostic [52].

Myosites observées au décours d'autres maladies auto-immunes systémiques

L'apparition d'une atteinte musculaire chez un malade ayant déjà un diagnostic de maladie auto-immune (notamment sclérodémie et SGS) n'est pas rare [53-56]. La sarcoïdose sera abordée au sein du chapitre relatif aux MG.

Scléromyosite

Une symptomatologie de myosite au cours d'une sclérodémie systémique a été rapportée dans plusieurs cohortes rassemblant plusieurs milliers de patients sclérodermiques avec des proportions variables (de 5 % à 60 %) [57-60]. Cette atteinte est majoritairement associée à la présence d'anticorps anti-PM/Scl (51 % en cas de positivité, *versus* 14 %). D'un point de vue histologique, une étude réalisée à partir de 26 cas rapporte l'association de lésions inflammatoires, d'une atrophie des fibres musculaires, de lésions de nécrose et régénération, et dans une moindre mesure, la présence de fibrose, et d'une vasculopathie [60]. Chez ces

patients souffrant de scléromyosite, la présence d'infiltrats inflammatoires et de nécrose à la biopsie, et la positivité des anticorps anti-PM/Scl semblent associées à une survie significativement meilleure [58]. Il est à noter qu'en cas de scléromyosite, d'autres auto-anticorps peuvent aussi être trouvés, notamment anti-U1RNP (15,7 %) et anti-Ku (2,3-55 %) [54, 56, 61].

Syndrome de Gougerot-Sjögren et myosite

La survenue d'une myosite au cours d'un SGS semble peu fréquente. Sa prévalence a été estimée lors de trois études et variait de 1 à 23 % [55, 62, 63]. Durant le SGS, une symptomatologie musculaire est plus fréquente sous forme de myalgies. Les cas associant SGS et myosite présentent une atteinte proximale symétrique, parfois associés à des atteintes axiales réalisant un syndrome de la tête tombante [64]. Lidvall *et al.* rapportent une forte proportion d'anomalies histologiques en cas de symptômes musculaires (72 %), néanmoins, seulement 14 % de ces lésions correspondaient à un aspect de myosite, dont 22 % ayant des similitudes avec le pattern anatomopathologique d'IBM, sans franche corrélation clinique [63]. La prévalence d'anticorps anti-Ro/SSA a été évaluée de 11 à 100 % des cas dans les myosites associées au SGS [54, 62]. L'atteinte pulmonaire commune aux deux pathologies doit être recherchée.

c. Myosite à inclusions

L'IBM est la MII la plus fréquente après 50 ans. L'âge moyen de son diagnostic est de 60-65 ans [65]. Le sexe ratio est de 2 hommes pour une femme. La prévalence varie entre 1 (Turquie) à 50 (Norvège, Australie)/million. Il s'agit d'une pathologie d'évolution lente et insidieuse, dont le tableau clinique complet n'apparaît qu'après plusieurs années d'évolution, ce qui explique la fréquence des diagnostics initiaux erronés (30-50 % de dystrophie musculaire, maladie du motoneurone, *etc.*). Cliniquement, l'atteinte est asymétrique et progressive. Un des premiers symptômes peut être la survenue de chutes récidivantes, avec une sensation de « lâchage du genou » rapportée par le patient. Apparaissent ensuite une faiblesse musculaire initialement proximale des membres inférieurs (quadriceps), puis une atteinte distale des membres supérieurs (fléchisseurs communs profonds des doigts et des poignets, les interosseux étant longtemps préservés), avec une atteinte très fréquemment plus importante du membre non dominant (Figure 6). L'atteinte distale des membres inférieurs, notamment des releveurs du pied est souvent plus tardive. La survenue de trouble de déglutition est fréquemment rapportée au cours de l'évolution de la maladie. Elle peut être isolée ou associée à une atteinte axiale à type de camptormie, et s'explique par un déficit de la contraction pharyngée, et un défaut de relaxation du sphincter supérieur de l'œsophage. Après plusieurs années, il existe typiquement une atteinte amyotrophique des quadriceps en « bouchon de champagne » (Figure 6), avec une réduction visible du volume du tiers inférieur de cuisse. De même, la survenue de rétractions des fléchisseurs doigts et des triceps suraux est fréquemment présente. Les myalgies sont rares, et le

taux de CPK est le plus souvent normal ou subnormal. Il est à noter qu'il n'est pas rare d'avoir des réflexes ostéotendineux diminués, et plus rarement une diplégie faciale [66]. L'atteinte des muscles oculaires est, elle, exceptionnelle. Les atteintes cardiopulmonaires sont rares, en dehors de pneumopathies d'inhalation en lien avec les troubles de déglutition. Plusieurs pathologies dysimmunitaires peuvent être associées à l'IBM, notamment le lupus systémique, le SGS, la sarcoïdose, la sclérodémie systémique, les déficits en immunoglobulines, ou encore une thrombopénie immuno-

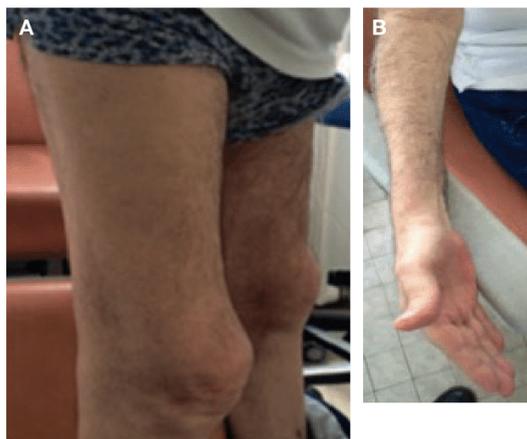


Figure 6 – Atteinte musculaire au décours d'une IBM. A. Amyotrophie quadricipitale en bouchon de champagne. B. Amyotrophie des fléchisseurs des doigts.

L'IRM met en évidence une sélectivité musculaire, avec une atteinte préférentielle du compartiment antérieur de cuisse (quadriceps, vastes latéraux). L'équipe de Tasca *et al.* ont défini un profil spécifique IBM avec l'involution et l'atrophie des muscles quadriceps et sartorius avec un gradient proximo-distale, associé à la préservation des muscles pelviens [72]. L'EMG met en évidence un tracé myogène, ou parfois mixte. Il est à noter qu'une neuropathie axonale modérée est parfois observée [73, 74].

L'analyse anatomopathologique est un point clef de la démarche diagnostique des IBM. Elle associe typiquement : 1) un élément inflammatoire composé d'infiltrats lymphocytaires T (CD8) et macrophagiques de l'endomysium, des lésions de myophagocytose de fibres non nécrotiques, et une hyperexpression du CMH-I au niveau sarcolemmal, 2) un élément vacuolaire avec dépôts de protéines amyloïdes (bêta-amyloïde, TDP43, p62) au sein des fibres, plus ou moins regroupés dans des vacuoles [75], 3) des anomalies mitochondriales, avec de trop nombreuses fibres COX négatives et SDH positives, 4) des lésions myopathiques avec notamment une irrégularité marquée de la taille des fibres. Même si cela est rare, des cas d'IBM présentant des granulomes non nécrotiques sur la biopsie musculaire ont été publiés [76, 77].

En 2013, l'anticorps anti-cN1A a été identifié comme un marqueur spécifique de la myosite à inclusions [78, 79]. Sa sensibilité varie de 37 à 76 % suivant les études, cependant, sa spécificité est bonne au sein des MII, puisque seulement 2 à 4 % des autres myosites étaient positifs. Néanmoins, cet anticorps a été rapporté au cours d'autres pathologies auto-immunes (20-37 % des SGS, 14-20 % des lupus) [80-82].

Le diagnostic est porté suivant les critères définis par Lloyd *et al.* en 2014 (sensibilité 90 %, spécificité 96 %) [83] :

– un critère clinique : atteinte des quadriceps et/ou des fléchisseurs des doigts ;

– deux critères histologiques : présence d'infiltrats inflammatoires endomysiaux sur la biopsie musculaire et présence de vacuoles bordées ou invasion de fibres non nécrotiques.

Néanmoins, de nombreuses discussions portent à l'heure actuelle sur une modification de ces critères au profit de critères cliniques permettant un diagnostic plus précoce [84, 85].

Les diagnostics différentiels comportent les formes familiales de myosite à inclusions, notamment la myopathie GNE autosomique récessive (dont un des éléments majeurs de différenciation est l'épargne quadricipitale visible à l'IRM) et la myopathie VCP autosomique dominante (dont la pénétrance est variable : cas familiaux de maladie de Paget, démence fronto-temporale, sclérose latérale amyotrophique, cataracte, cardiomyopathie dilatée). Malgré l'étiologie génétique, un infiltrat inflammatoire modeste peut parfois être retrouvé [86, 87].

D'un point de vue thérapeutique, aucun traitement n'a fait à ce jour la preuve de son efficacité en dehors d'une kinésithérapie pluri-hebdomadaire [88, 89]. Des petites doses de corticoïdes peuvent être bénéfiques, avec néanmoins le risque non négligeable du développement d'une myopathie cortisonique. En cas de troubles majeurs de la déglutition, un geste chirurgical de myotomie cricopharyngée peut être réalisé, même si le bénéfice clinique s'estompe en quelques années. Dans ce cas particulier, l'indication de perfusion d'immunoglobulines polyvalentes (IgIV) peut être discutée. L'évolution est donc lente et progressive, avec la perte de la marche en 10-15 ans, sans modification de la survie des malades.

3. Myosites rares

En dehors des groupes de MII bien connus, d'autres entités rares peuvent être rencontrées parmi lesquelles les myosites secondaires aux immunothérapies (MSI), les myosites granulomateuses (MG) et les myosites focales (MF). Leurs phénotypes cliniques, histologiques et évolutifs sont variables, rendant parfois le diagnostic difficile, et modulant leurs prises en charge.

a. Myosites secondaires aux inhibiteurs de checkpoint (MSI)

L'immunothérapie anticancéreuse, utilisant les inhibiteurs de checkpoint (notamment, anti-CTLA4 : ipilimumab, anti-PD1 : nivolumab et anti-PDL1 : atezolizumab) a révolutionné la prise en charge de certaines néoplasies au

pronostic sombre (voir chapitre « Maladies systémiques d'origine toxique et médicamenteuse », vol. 2). Des effets secondaires sévères dus à l'émergence de clones T auto-réactifs et actifs ont été rapportés [90, 91], sous forme d'atteintes auto-immunes : entérites, dermatites, hépatites, pneumopathies, hypophysites. Des observations atteignant l'appareil musculosquelettique ont aussi été rapportées, définissant ainsi une nouvelle entité au sein du groupe des MII : la MSI.

Dans une large analyse des bases de données colligeant plus de 20000 patients traités par ipilimumab et/ou nivolumab (Bristol-Myers Squibb corporate safety databases), une myosite était identifiée chez 0,2 % des patients recevant l'association des 2 inhibiteurs de checkpoint, et de 0,1 % en cas d'utilisation de nivolumab seul.

Le développement de la MSI fait généralement suite à la première administration, et cela dans un délai médian d'une quinzaine de jours. Un tableau de dermatomyosite typique a été rapporté [92], ainsi que des cas présentant des atteintes axiales et proximo-distales [93, 94]. Tous présentaient une élévation des CPK. Chez des patients recevant de lourds traitements anticancéreux, la prise en charge thérapeutique est complexe. Dans les cas rapportés, des traitements par IgIV et corticothérapie ont été mis en place, et l'inhibiteur de checkpoint arrêté. La publication récente de 2 cas ayant présenté des tableaux de rhabdomyolyses, myosites et myocardites fulminantes létales souligne la nécessité de ne pas méconnaître ces tableaux [95]. De manière surprenante, l'infiltrat du myocarde, du muscle squelettique et de la tumeur semblait être de composition similaire (population lymphocytaire T clonale).

b. Myosites granulomateuses

Entité très rare, la MG est définie sur le plan anatomopathologique, par la présence de granulomes inflammatoires sans nécrose caséuse, souvent associés à des cellules géantes, au sein du muscle squelettique (voir chapitre « Granulomatoses », vol. 1). Sa prévalence a été évaluée à 0,5 % dans une large série de 2985 biopsies musculaires [96], sans distinction de diagnostics étiologiques ou syndromiques. Plusieurs petites séries de cas relatent leurs expressions cliniques et leurs associations à différentes pathologies. L'âge moyen de survenue est d'environ 60 ans [97, 98]. Les MG peuvent être isolées, ou s'intégrer dans d'autres cadres pathologiques, en premier lieu, la sarcoïdose, mais aussi la maladie de Crohn, les lymphomes, les thymomes, la myasthénie, une réaction à un corps étranger, ou encore des maladies infectieuses [99, 100]. Les différents travaux publiés identifient deux profils assez différents : les MG isolées (MGI) et les MG dans le cadre de sarcoïdose (MGS) [97, 98, 101].

Les MGS surviennent au cours de sarcoïdose connue dans la majorité des cas, et se présentent sous la forme d'une atteinte proximale et symétrique sévère touchant majoritairement les membres inférieurs, associée à des lésions anatomopathologiques compatibles. Dans ces cas, la survenue d'une dysphagie est inconstante.

La simple présence de granulomes sur la biopsie musculaire, sans atteinte clinique, n'est pas prédictive de l'apparition d'une myopathie. En effet, suivant les études, 50 à

80 % des patients atteints de sarcoïdose présentaient ce type de lésions histologiques musculaires, sans corrélation clinique évidente, puisque seul 1,4-2,3 % des patients étaient symptomatiques [102-104]. Les atteintes musculaires spécifiques de la sarcoïdose ont été classées en trois groupes : nodules musculaires palpables, myosite aiguë de survenue précoce (potentiellement compliquée d'hypercalcémies sévères) et myopathie chronique de survenue tardive qui correspond aux MGS [103, 105, 106].

Les MGI, sont quant à elle d'évolution plus lente. Elles se présentent plus fréquemment sous forme d'atteinte distale des membres supérieurs, possiblement associées à une dysphagie. Plusieurs publications relatent l'existence de lésions granulomateuses non nécrosantes sur des biopsies musculaires de cas d'IBM [76, 77, 107]. La possibilité d'une certaine intrication entre MGI et IBM pourrait par ailleurs être étayée par les similitudes cliniques (atteintes distales et dysphagie) et physiopathologiques (signature TH1) [108, 109]. Néanmoins les faibles effectifs invitent à la prudence dans l'interprétation de ces données.

Dans les MGS comme les MGI, les CPK restent le plus souvent normales ou très modérément élevées. L'imagerie trouve sa place habituelle dans la mise en évidence des muscles atteints, avec lésions inflammatoires et atrophie musculaire. Un *case report* montre l'utilité de la scintigraphie au gallium 67, avec la mise en évidence d'une prise de contraste nodulaire [110]. Au cours de la sarcoïdose, le PET scanner peut mettre en évidence une atteinte musculaire diffuse ou orienter le diagnostic avec la mise en évidence de fixations ganglionnaires médiastinales ou des glandes salivaires [111, 112].

Aux vues de ces éléments, la MG semble plutôt être à considérer comme un syndrome, pouvant correspondre à une atteinte réactionnelle (micro-organismes, cancers, dysimmunité et corps étranger), une atteinte s'intégrant dans le cadre d'une sarcoïdose, ou une atteinte isolée [113]. La découverte de granulome non nécrotique sur une biopsie musculaire doit néanmoins inciter le clinicien à faire un bilan complet dans le but d'éliminer des diagnostics associés graves ou curables (Tableau 2).

- Sarcoïdose
- Maladie infectieuse :
 - bactérienne : tuberculose, syphilis, brucellose, Lyme, maladie de Whipple, lèpre
 - virale : VIH, HTLV-1
 - parasitaire : toxoplasmose, sporotrichose
 - mycose : cryptococcose, candidose
- Hémopathie : lymphome
- Thymome (associé ou non à une myasthénie)
- Tumeurs solides : mélanome, carcinome mammaire, dysgerminome suprasellaire
- Association à d'autres maladies dysimmunitaires : cirrhose biliaire primitive, myasthénie, maladie de Crohn
- Vascularite : granulomateuse avec polyangéite, granulomateuse éosinophilique avec polyangéite
- Connectivites : DM, fasciite à éosinophiles, polyarthrite rhumatoïde, Gougerot-Sjögren, lupus
- Réaction induite par la présence de corps étrangers : béryllose, implants mammaires en silicone

Tableau 2 – Diagnostics à rechercher en cas de granulomes à la biopsie musculaire.

c. Myosite focale

Décrite pour la première fois en 1977 par Heffner *et al.*, la MF est une maladie rare appartenant au groupe des MII [114]. Le diagnostic repose sur un faisceau d'arguments comprenant une clinique et une évolution compatibles, la présence d'anomalies localisées sur les examens morphologiques, électrophysiologiques et histologiques, et l'absence d'anomalie à distance [115].

À ce jour, seulement 250 cas ont été rapportés dans la littérature, la plupart publiés sous forme de cas isolés ou de petites séries comptant de 4 à 16 patients [116-118]. Une seule étude a été réalisée à partir d'une large cohorte ($n = 115$) par Auerbach *et al.*, qui ont réalisé un important travail de description des lésions anatomopathologiques macroscopiques et microscopiques [119].

Cliniquement, la MF peut survenir à tous les âges, même si elle semble surtout toucher l'adulte dans la quatrième décennie. Cliniquement, la présentation est celle d'une masse inflammatoire isolée, développée aux dépens du tissu musculaire squelettique. Le caractère unifocal n'est pour autant pas systématique. De fait, Heffner *et al.* ont décrit des lésions affectant plus de 2 à 3 muscles dans un peu plus d'un tiers des cas. À la différence des autres MII, les tableaux cliniques restent cependant circonscrits. D'autres séries de cas s'accordent avec la possibilité d'une atteinte pluri-monofocale, notamment remarquable lors des investigations morphologiques [117, 118, 120]. La localisation de la MF est variable, atteignant les membres inférieurs dans une majorité de cas. Pour autant tous les muscles semblent pouvoir être atteints (membres supérieurs, muscles paravertébraux, muscles cricopharyngés, langue, muscle de l'orbite, *etc.*) comme l'atteste l'analyse de la littérature [121]. La MF est une pathologie le plus souvent localement douloureuse, sans faiblesse musculaire en dehors d'une impotence fonctionnelle algique. L'association à des symptômes généraux semble rare. Le taux de CPK est rapporté comme habituellement normal ou très modérément élevé, de même que les marqueurs inflammatoires (CRP, vitesse de sédimentation notamment). À l'heure actuelle, aucun auto-anticorps spécifique n'a été identifié. Les examens morphologiques (IRM mettant en évidence des lésions inflammatoires iso intense en T1 et hyperintense sur les séquences T2 [Figure 7]) et électrophysiologiques permettent de confirmer le caractère focalisé de l'atteinte myogène. L'analyse anatomopathologique met en évidence un profil de myosite aspécifique qui est circonscrit à un muscle, et comporte l'association d'éléments myogéniques (anisodiamétrie, fibrose) et myositiques (infiltrats inflammatoires endomysiaux et périnmysiaux, expression sarcolemmale du CMH-I positive faible) auxquels s'ajoutent parfois des éléments neurogéniques (sacs nucléaires).

La pathologie a une évolution aiguë ou subaiguë, régressant habituellement spontanément. L'histoire de la maladie peut néanmoins s'émailler de récurrences dont la localisation peut être similaire ou varier [122, 123]. Il n'existe pas à l'heure actuelle de consensus pour la prise en charge thérapeutique. Dans la littérature, pour les cas particulièrement douloureux, ou récurrents, l'utilisation de corticoïdes

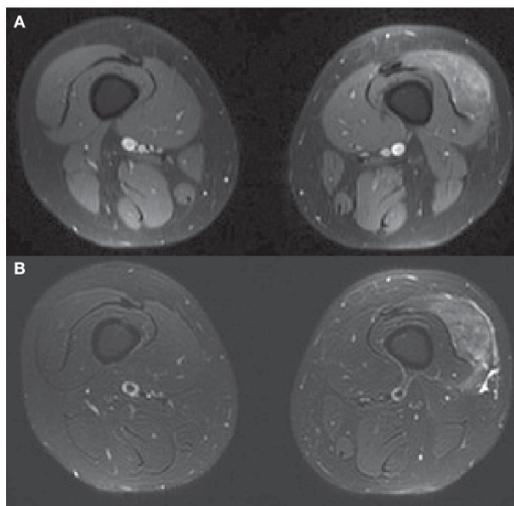
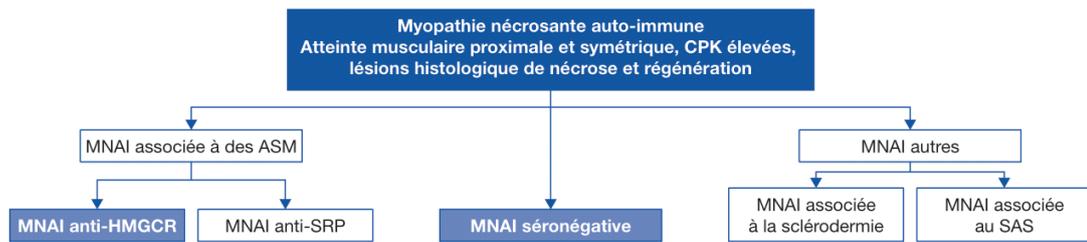


Figure 7 – IRM musculaire au décours d'une myosite focale. A. Hypersignal T1 focal avec prise de gadolinium du vaste latéral gauche. B. Hypersignal T2 focal du vaste latéral gauche.

apporte un bénéfice variable [124-126]. Les immunosuppresseurs utilisés en cas de MF réfractaires ont une efficacité partielle [121]. LMF peut être associée à d'autres pathologies, comme des radiculopathies [124, 125], des maladies infectieuses comme la maladie de Lyme [126], ou encore des pathologies dysimmunitaires (maladie de Behçet) [128, 129]. De rares cas d'association à des néoplasies ont été publiés, incitant à être vigilant lors de la prise en charge de cette pathologie [130-132].

B. MYOPATHIES NÉCROSANTES AUTO-IMMUNES

Définies en 2004 dans les classifications internationales, les myopathies nécrosantes auto-immunes (MNAI) appartiennent au groupe des MII dont elles représentent jusqu'à 20 % des cas [3, 133]. Les MNAI comprennent plusieurs entités : les MNAI associées à des ASM (anti-HMGCR et anti-SRP), les MNAI séronégatives, les MNAI associées à des maladies systémiques (notamment la sclérodémie systémique), et les MNAI associées à un anticorps antisynthétase (Figure 8) [134]. Elles se définissent par l'association d'une atteinte musculaire proximale symétrique atteignant les ceintures pelviennes et scapulaires, avec des CPK élevées (en général $> 1\,000$ UI/L) et des lésions histologiques de nécrose/régénération avec peu ou pas d'infiltrats inflammatoires. Malgré l'absence d'infiltrat retrouvé habituellement dans les myosites, leur statut de maladie dysimmunitaire est corroboré par la présence d'ASM identifiés, les éléments histologiques (dépôt de complexe d'attaque membranaire (C5b9) au niveau sarcolemmal, présence de quelques infiltrats de cellules immunitaires à distance des foyers de nécrose), et par leurs réponses aux traitements immunomodulateurs.



ASM : auto-anticorps spécifique des myopathie idiopathique inflammatoire (MII) ; AAM : auto-anticorps associé aux MII ; MNAI : myopathie nécrosante auto-immune ; SAS : syndrome des antisynthèses. Surlignage bleu clair : MNAI identifiées comme fréquemment associées aux cancers.

Figure 8 – Différents sous-groupes dans les myopathies nécrosantes auto-immunes.

Les MNAI peuvent toucher tous les âges même si les cas pédiatriques restent rares. L'atteinte musculaire est le plus souvent isolée. Il n'y a pas de corrélation stricte entre taux de CPK et nombre de fibres en nécrose. Même si la présentation clinique est le plus souvent aiguë ou rapidement progressive, des formes d'évolution plus lentes ont été rapportées. Cela, ajouté à l'atteinte anatomopathologique peu inflammatoire, peut être responsable d'errance diagnostique de plusieurs années [135-137]. Les principaux diagnostics différentiels sont alors les dystrophies musculaires des ceintures dont la dysferlinopathie, l'anocaminopathie, et l' α -dystroglycanopathie, des atteintes toxiques et infectieuses [138]. Les antécédents familiaux de maladies neuromusculaires, l'atteinte faciale, l'atteinte musculaire asymétrique, la dysphagie, la présence d'activité spontanée à l'électromyogramme, ainsi que la présence d'anomalies anatomopathologiques spécifiques (marquage de la dysferline par exemple) sont autant d'éléments à prendre en compte dans la démarche diagnostique.

1. Myopathies nécrosantes associée à des auto-anticorps

Deux auto-anticorps ont été identifiés au cours des MNAI : les anti-HMGCR (*hydroxymethylglutaryl-coenzymeA-reductase*), et les anti-SRP (*Signal Recognition Particle*). L'étude des MNAI associées à ces anticorps a permis d'individualiser des sous-groupes dont le phénotype clinique et l'évolution diffèrent, impliquant des variations dans la prise en charge. À l'examen histologique, on trouve des fibres en nécrose et en régénération en quantité importante, réparties de manière aléatoire dans la biopsie. Le CMH-I est surexprimé sur le pourtour des fibres, avec des foyers hyperintenses autour des zones de myophagocytose. Des dépôts de complément sont retrouvés au niveau sarcolemmal. La présence d'infiltrats inflammatoires est rare [134]. Concernant les données immunologiques, il est intéressant de souligner que plusieurs études ont rapporté une corrélation entre le taux de ces auto-anticorps et l'activité de la maladie [139, 140].

a. MNAI anti-SRP

SRP (*Signal Recognition Particle*) est un complexe de protéine et d'ARN ayant pour fonction de fixer les ribosomes

sur le réticulum endoplasmique, jouant ainsi un rôle dans l'adressage des protéines dans la cellule. L'anticorps anti-SRP a été décrit pour la première fois par Reeves *et al.* en 1986 [141]. Il est rapporté dans 3 à 6 % des MII, et plus de 15 % des MNAI [31, 142]. Cliniquement, les MNAI à anti-SRP surviennent le plus souvent chez l'adulte jeune (3^e décennie), avec une prédominance féminine [143]. De rares cas pédiatriques ont été rapportés [136, 144]. Concernant les facteurs de risque de la maladie, une étude a fait part d'une association aux groupages HLA B*5001 et DQA1*0104 [145]. Cliniquement, l'atteinte musculaire est aiguë, avec une atteinte proximale d'emblée sévère, associée à une dysphagie dans plus d'un tiers des cas. Dans une étude de 39 cas, la prévalence des atteintes pulmonaires interstitielles était de 22 %, incitant à ne pas négliger ce point dans la prise en charge de la pathologie [143]. La survenue d'atteintes cardiaques (cardiopathies rythmiques, cardiomyopathies dilatées), parfois graves a été rapportée par certains auteurs [146, 147]. Les autres atteintes extramusculaires, notamment articulaires et cutanées restent rares. Le taux de CPK est élevé de manière constante, et semble proportionnel à la faiblesse musculaire. Contrairement aux MNAI anti-HMGCR et séronégatives, les MNAI anti-SRP ne sont pas associées aux cancers.

Il s'agit d'une pathologie potentiellement sévère : dans leur étude, Pinal Fernandez *et al.* notent la persistance des symptômes musculaires à 4 ans dans un cas sur deux, et ce quel que soit le traitement réalisé. Certains patients semblent plus réfractaires, notamment en cas de diagnostic initial porté chez un patient jeune, avec une faiblesse musculaire sévère, et un taux de CPK > 5000 UI/L. En l'absence de consensus, les IgIV seules, ou associées à des immunosuppresseurs (corticoïdes et méthotrexate) sont utilisés. Il a été rapporté une relative efficacité rétrospective du rituximab dans 13 cas sur 17 [143]. Considérant la pathogénicité démontrée des auto-anticorps, la place des échanges plasmatiques en cas de gravité peut être envisagée [148]. Néanmoins, ces données nécessitent des études prospectives pour être validées.

b. MNAI anti-HMGCR

HMGCR (*hydroxymethylglutaryl-coenzymeA-reductase*) est une enzyme intervenant dans les voies métaboliques de

différents lipides, notamment le cholestérol et les terpénoïdes. Les statines, médicaments les plus utilisés à l'heure actuelle dans la prévention du risque cardiovasculaire, sont des inhibiteurs de la HMGCR. L'anti-HMGCR a été décrit en 2010 par Christopher-Stine *et al.* [142]. Une exposition préalable aux statines (médicaments ou statines végétales) est rapportée dans 68 à 83 % des cas [135, 149-151]. Il semble important de rappeler que l'intolérance aux statines qui associe des myalgies et une légère augmentation des CPK ne correspond qu'exceptionnellement à une MNAI (*cf.* paragraphe myopathie toxique). Afin de différencier ces deux atteintes, un testing musculaire, et un bilan paraclinique orienté, notamment le dosage de l'anti-HMGCR dont la spécificité est forte, sont nécessaires [152]. Au cours de la MNAI avec anti-HMGCR, l'arrêt des hypolipémiants n'influence généralement pas son évolution [153]. La présence de l'antigène HLA de classe 2, allèle DRB1*11:01 [154] est associé au développement de la maladie. Néanmoins, il paraît évident que d'autres facteurs rentrent en jeu dans la pathogénie, expliquant la faible incidence de la pathologie par rapport à la forte prévalence de ce sous-type HLA et de la prescription de statines dans la population générale.

Cliniquement, les MNAI anti-HMGCR surviennent en général à l'âge adulte. Quelques rares cas pédiatriques ont été identifiés, représentant 1 % des myosites juvéniles [155]. L'atteinte musculaire semble plus modérée que dans les MNAI à anti-SRP, et les atteintes extramusculaires, notamment pulmonaires sont très rares [143, 151].

D'un point de vue histologique, le pattern de nécrose/régénération habituel est retrouvé, avec une surexpression du CHM-I au niveau sarcolemmal. Des dépôts de C5b9 sont retrouvés fréquemment, contrairement aux infiltrats inflammatoires endomysiaux et périvasculaires (20-38 % des cas). La présence d'infiltrats inflammatoires semble être plus fréquente en cas d'exposition préalable aux statines [149, 156].

Cette pathologie présente souvent une évolution longue avec des rechutes fréquentes à l'arrêt des traitements (jusqu'à 60 % des cas) [150, 151]. Les IgIV, seules, ou en association avec un traitement immunosuppresseur pouvant utiliser plusieurs molécules (corticothérapie, méthotrexate, azathioprine) sont généralement utilisés, malgré l'absence d'essai thérapeutique [157, 158]. Comme dans les MNAI avec anti-SRP, la place des échanges plasmatiques dans cette pathologie à médiation humorale nécessite d'être définie [148]. De manière intéressante, chez les patients jeunes, l'atteinte apparaît comme plus sévère et plus difficile à traiter [159], alors que chez les plus de 65 ans, elle est rapportée d'évolution plus favorable, notamment en cas d'exposition aux statines, à l'exception des cas associés à un cancer.

Allenbach *et al.* ont rapporté la survenue d'une néoplasie dans les 3 années précédant ou suivant le diagnostic chez 17,3 % des MNAI anti-HMGCR, avec 11,5 % des patients ayant une néoplasie synchrone [160]. Plus anecdotique, l'anti-HMGCR est retrouvé dans la sclérodermie systémique (fréquence 1,3 %) sans corrélation exacte avec la présence d'une atteinte musculaire type MNAI.

2. Myopathies nécrosantes séronégatives (MNAI ASM-)

Certains patients présentent un tableau typique de MNAI, avec la triade clinique, biologique et anatomopathologique compatible, sans pour autant que l'on puisse identifier d'ASM. Ces cas sont relativement similaires aux MNAI associées aux anti-SRP et HMGCR, avec une moyenne d'âge au diagnostic autour de la cinquième décennie. L'atteinte musculaire est isolée, et semble aussi sévère que dans les MNAI anti-SRP. Le taux de CPK est parfois très élevé. L'examen anatomopathologique met en évidence un muscle très altéré avec de nombreuses fibres en nécrose [160]. Le point crucial de la prise en charge des MNAI ASM- est la réalisation d'un bilan exhaustif à la recherche d'un cancer. Décrites en 1998 par Levin *et al.*, les myopathies nécrosantes satellites d'un cancer sont à intégrer dans le sous-groupe MNAI ASM- [161]. En effet, dans une large étude des cas de myosites paranéoplasiques, Allenbach *et al.* rapportaient une néoplasie dans les 3 années précédant ou suivant le diagnostic chez 28,6 % des MNAI séronégatives, le *standardized incident ratio* s'élevant alors à 8,35 par rapport à la population générale. Encore plus remarquable, le cancer était synchrone chez 21,4 % des patients. L'anatomopathologie des tumeurs est variable comprenant principalement des carcinomes digestifs et des lymphomes [160].

3. Myopathies nécrosantes autres

a. MNAI associées aux maladies systémiques

L'analyse anatomopathologique de patients ayant des atteintes musculaires inflammatoires dans le cadre de maladies de système révèle parfois des lésions de nécrose en proportion importante. Ci-après sont relatées les quelques données sur ces rares associations.

MNAI et connectivites

Comme décrit dans le paragraphe relatif aux OM, la survenue d'une MII au décours de la sclérodermie systémique est possible. Il s'agit le plus souvent de myosites associées à des AAM (anti-PM/Scl, U1-RNP, Ku), avec un infiltrat inflammatoire prédominant et une surexpression du CMH-I. Néanmoins, l'analyse, par Ranque *et al.* d'une cohorte de 35 patients sclérodermiques ayant des symptômes et exploration musculaires a permis de mettre en évidence des lésions histologiques de nécrose et régénération dans 59 % des cas [60]. Pour autant, les lésions de nécrose étaient souvent associées à une inflammation, et corrélées à une évolution favorable, à la différence des atteintes musculaires dont la biopsie ne retrouvait pas d'élément en faveur de MII. Un traitement par corticothérapie semblait favorable chez les cas avec lésions histologiques très inflammatoires. Le risque de crise rénale sclérodermique lors de l'administration de corticoïdes à haute dose doit toutefois être pris en considération. En l'absence de consensus, cette décision thérapeutique doit être évaluée au cas par cas en fonction des facteurs de risque usuels de crise rénale [162].

MNAI et SAS

La présence de lésions de nécrose sur la biopsie musculaire des patients ayant un SAS est possible. En effet, Christopher-Stine *et al.* ont rapporté un diagnostic de SAS pour quatre patients d’une cohorte de 38 patients ayant une myosite nécrosante [138]. Les fibres en nécrose ont ici une distribution essentiellement périfasciculaire et non endomyosiale comme dans les MNAI anti-SRP ou HMGCR+ [163]. Cette donnée est à considérer dans la démarche diagnostique devant une myopathie nécrosante, et rappelle la nécessité de rechercher les éléments cliniques extramusculaires, ainsi que la présence d’ASM.

4. Les atteintes extramusculaires graves

a. MII et cancer (Figure 9)

L’association MII et cancer ne doit pas être méconnue, eu égard à la nécessité de diagnostic carcinologique le plus précoce possible. Certains sous-groupes sont significativement associés à la survenue d’un cancer, notamment les DM anti-TIF1γ, DM anti-NXP2, MNAI anti-HMGCR, MNAI ASM- et les MF [11, 21, 30, 160, 164]. Des facteurs de risques de cancer ont été identifiés (Tableau 3) et sont détaillés ci-après. Cliniquement : l’âge de plus de 50 ans [165, 166], ou présence d’une dysphagie [165, 167] sont rapportés comme significativement associées au cancer. Biologiquement, la valeur des CPK est discutée par plusieurs auteurs, sans claire association dans l’entité globale MII [167]. Au cours des DM, des études spécifiques ont identifié d’autres facteurs de risque de cancer : un début des symptômes musculaires ou cutanés rapide, une atteinte cutanée sévère (ulcérations et nécroses cutanées souvent résistantes au traitement, ainsi que l’érythème péri-unguéal), et un syndrome inflammatoire au diagnostic (VS > 35 mm) [168-170]. La présence d’une lymphopénie < 1 500/mm³ a quant à elle été retrouvée comme facteur protecteur [168]. La survenue du cancer intervient le plus souvent dans l’année suivant le diagnostic, et dans une moindre mesure les 3 à 5 ans suivant le diagnostic de MII [167, 171]. En cas de diagnostic d’un des sous-groupes suscités, un bilan initial exhaustif doit donc être réalisé à la recherche d’une néoplasie solide ou hémato-logique. Le bilan doit être orienté par la clinique et associée : scanner thoraco-abdomino-pelvien, fibroscopie gastrique, colonoscopie (âge > 60 ans), examens gynécologiques avec échographie pelvienne et mammographie chez la femme, examen urologique et PSA chez l’homme, voire PET scanner. Il est à noter que le PET scanner n’a pas montré sa supériorité par rapport au bilan oncologique conventionnel [172]. Les marqueurs tumoraux, notamment CA 125, et dans une moindre mesure, le CA 19-9, peuvent également être considéré dans l’évaluation du risque de cancer associé [173]. Une étude épidémiologique récente souligne l’importance de rechercher activement le cancer la première année puis d’étendre la vigilance 3 à 5 ans en fonction de l’évolution de la maladie [170, 171]. Des facteurs «protecteurs ont aussi été identifiés : existence d’une atteinte pulmonaire interstitielle, d’une

atteinte articulaire (arthrite ou arthralgies) et anticorps anti-Jo-1 [167].

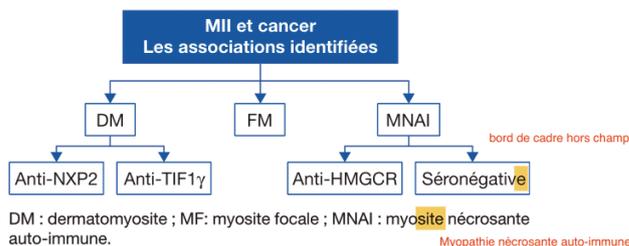


Figure 9 – Myopathies inflammatoires idiopathiques (MII) et cancer.

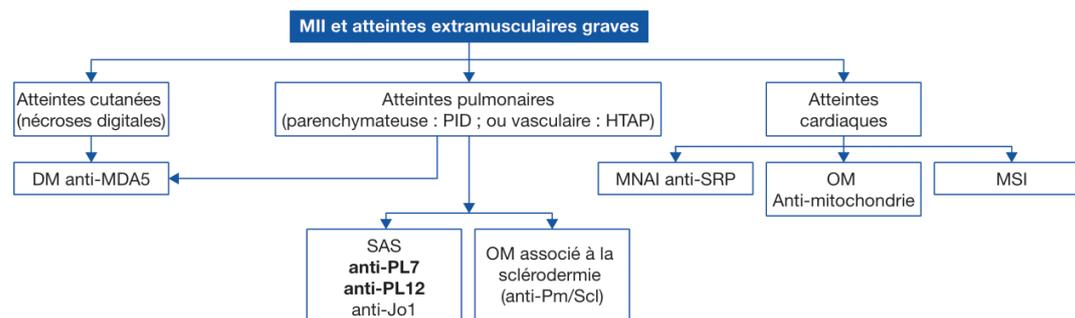
Facteurs de risque	Facteurs protecteurs
Âge > 50 ans	PID
<ul style="list-style-type: none"> • Début rapide • Nécrose cutanée • Dysphagie • Baisse du C4 • Lymphocytopénie • Résistance aux traitements 	<ul style="list-style-type: none"> • Arthrites et arthralgies • Anti Jo-1
DM à anti-TIF1g	
DM à anti-NXP2	
MNAI à anti-HMGCR MNAI Séronégatives	

DM : dermatomyosite ; MNAI : myosite nécrosante auto-immune ; PID : pneumopathie interstitielle diffuse.

Tableau 3 – Facteurs prédictifs de cancer lors des IIM.

b. Atteintes cardiaques ou pulmonaires graves à dépister (Figure 10)

Certains sous-groupes de MII sont particulièrement associés à des atteintes extramusculaires graves. Au sein des DM, seules les DM associées aux anti-MDA5 présentent des atteintes potentiellement graves, dont les nécroses cutanées, et les lésions ulcérées à l’emporte-pièce constituent de véritables portes d’entrées infectieuses d’autant que les patients sont immunodéprimés. L’atteinte pulmonaire (parenchymateuse et/ou vasculaire) est identifiée dans de nombreuses MII, dont les SAS, certaines DM et plusieurs OM (anti-Ku, PM/Scl, etc.). Elle est particulièrement sévère et d’évolution rapide dans les DM anti-MDA5, le SAS avec anti-PL7 ou PL12, et les OM type scléromyosite. L’atteinte cardiaque reste relativement rare. En dehors des atteintes cardiaques droites compliquant les HTAP des anti-MDA5, des SAS et des scléromyosites, des atteintes myocardiques potentiellement graves peuvent survenir dans les MNAI anti-SRP, MSI et les OM anti-mitochondrie.



DM : dermatomyosite ; MSI : myosite secondaire aux inhibiteurs de checkpoint ; OM : overlap myositis ou myosite de chevauchement ; SAS : syndrome des anti-synthétases ; PID : pneumopathie interstitielle diffuse ; HTAP : hypertension artérielle pulmonaire.

Figure 10 – Myopathies inflammatoires idiopathiques (MII) et atteintes extramusculaires graves.

MYOPATHIES TOXIQUES, IATROGÈNES, ENDOCRINIENNES

A. MYOPATHIES IATROGÈNES ET TOXIQUES

Le muscle peut être détérioré par des substances dites myotoxiques au premier rang desquelles se trouvent les substances prises dans le cadre ou en dehors de la prescription médicale. Cette toxicité peut se faire par plusieurs biais : 1) l'altération d'organelles des fibres musculaires (mitochondrie, lysosome, myofibrille), 2) une perturbation de l'homéostasie électrolytique, 3) l'absorption des ressources indispensables au fonctionnement musculaire et 4) l'altération des antigènes musculaires induisant une réaction immunologique ciblant la fibre musculaire. Dans ce dernier cas, il s'agira de myosite iatrogène et non de myopathie iatrogène ou toxique (cf. paragraphe myosites secondaires aux inhibiteurs de checkpoint). Cliniquement, un élément majeur est la séquence temporelle compatible. En dehors des myopathies aiguës de réanimation, des rhabdomyolyses post-anesthésiques sur terrain permissif (hyperthermie maligne induite lors de l'injection de certains agents anesthésiques, notamment l'halotane et la succinylcholine) ou des intoxications éthyliques massives, l'ensemble des tableaux de myopathies iatrogènes ou toxiques survient de manière subaiguë, voire chronique. Les patients présentent des symptômes myopathiques avec une fatigue, des myalgies, une faiblesse musculaire proximale symétrique, parfois, une myoglobulinurie et des CPK augmentées. Les lésions anatomopathologiques dépendent du mécanisme de myotoxicité sous-jacent. Dans la majorité des cas, si le diagnostic est évoqué précocement, un arrêt de la drogue imputée entraîne une amélioration du tableau, étayant ainsi le lien causatif.

Parce qu'il s'agit de pathologies potentiellement réversibles, un diagnostic précoce, un relais d'information auprès de la pharmacovigilance, ainsi qu'un traitement rapide du malade sont indispensables.

1. Myopathies iatrogènes

Dans nos pratiques habituelles de médecins internistes, plusieurs molécules peuvent être responsables de myopathies iatrogènes (hydroxychloroquine, colchicine). La présentation clinique est classique et leur classification repose sur les mécanismes pathogéniques et les lésions anatomopathologiques qui en résultent [174].

La **myopathie cortisonique** est une myopathie avec atrophie de fibre de type II. Elle survient lors d'une prescription prolongée de corticostéroïde et est favorisée par l'absence d'exercice physique. L'atteinte est progressive, le taux de CPK reste en général normal. L'analyse histologique montre une diminution de la taille des fibres de type 2, alors que les fibres de type 1 ont une taille conservée. L'arrêt du traitement et l'activité physique permettent la stabilisation voire une récupération complète.

Des myopathies mitochondriales sont possibles lors des traitements par **analogues nucléosidiques**, notamment la zidovudine (AZT) et la lamivudine (3TC). La fatigabilité et les myalgies surviennent après une exposition de 6 à 12 mois. Les CPK sont fréquemment augmentées. L'analyse histologique révèle des fibres rouges déchetées (*Ragged-Red Fibers* [RRF]), des fibres COX négatives en trop grand nombre pour l'âge, et parfois des accumulations lipidiques au sein des fibres. L'arrêt du traitement en cause n'améliore pas toujours la symptomatologie.

Des myopathies lysosomales peuvent être causées par les antipaludéens de synthèse, au premier rang desquels la chloroquine et l'**hydroxychloroquine**. Dans une étude prospective de 2005, Casado *et al.* identifiaient 15 patients (13 %) ayant des anomalies musculaires (CPK, histologie) au sein d'une cohorte de 119 malades recevant dans antipaludéens de synthèse pour une durée minimale de 6 mois [175]. Ces molécules, par leur caractère amphiphile, sont capables d'interagir avec les phospholipides membranaires, induisant la formation pathologique de vacuoles autophagiques que la cellule musculaire n'arrive pas à éliminer. Les patients présentent ce type d'atteinte après une administration à forte dose (500 mg/jour) et au long cours. La

symptomatologie musculaire classique peut se compléter par une atteinte des muscles respiratoires et cardiaques, et parfois d'une neuropathie sensitive [176, 177]. Les CPK sont en général normales ou subnormales. L'analyse histologique montre une atteinte semblable aux lésions retrouvées dans la maladie de Pompe, avec de multiples vacuoles marquées par la phosphatase acide, et la présence importante de lysosomes de taille augmentée [178]. La récupération après arrêt du traitement est lente.

Des myopathies vacuolaires par dysfonction des microtubules sont rapportées en cas de prescription au long cours de **colchicine**. En effet, cette molécule perturbe la polymérisation des microtubules. Cela aboutit à une sollicitation importante des processus de nettoyage de la cellule musculaire que sont les voies de dégradation autophagique et lysosomale. Il en découle un défaut d'élimination des déchets cellulaires. Les patients ont en général entre 50-70 ans, des antécédents de goutte et d'insuffisance rénale modérée. Le phénotype clinique est classique et peut s'associer à une neuropathie axonale avec aréflexie [179, 180]. Le taux de CPK est habituellement augmenté. L'atteinte s'améliore 4-6 semaines après l'arrêt de la colchicine.

Des myopathies myofibrillaires sont rapportées dans le cadre de traitement par l'**émétine**. En effet, cet antiparasitaire est responsable de perturbations de la strie-Z, induisant une accumulation de myofibrilles désorganisées au sein de la fibre musculaire [174].

Les myopathies toxiques nécrosantes associées aux **statines** et aux fibrates sont des entités rares, dont l'incidence est de 2 à 3 patients pour 100 000 patients traités par statines [151]. Elles sont dues à un mécanisme mettant en jeu le cytochrome P450-3A4 et se traduisent par une l'hyperCKémie asymptomatique, une hyperCKémie avec myalgies ou une rhabdomyolyse. Dans les deux premiers cas, l'analyse histologique montre quelques lésions nécrotiques et quelques fibres rouges déchiquetées. Contrairement aux MII, la symptomatologie est régressive à l'interruption du traitement. La fréquence des rhabdomyolyses est évaluée à 1/100 000 patients traités par statine. Elles surviennent en général dans un contexte de prise concomitante de traitements interagissant avec le cytochrome P450-3A4, tels que la ciclosporine, l'amiodarone, les macrolides, l'acide fucidique (association contre-indiquée), le vérapamil ou le gemfibrozil.

Enfin, le **syndrome malin des neuroleptiques** est une situation qui ne correspond pas exactement à une myopathie, mais implique le muscle via d'autres mécanismes. En effet, les neuroleptiques induisent un blocage des récepteurs sérotoninergiques, responsable d'une contraction non contrôlée des fibres musculaires se compliquant de myolyse.

2. Myopathies toxiques

La prise de substance telle que l'alcool, les amphétamines, la cocaïne et l'héroïne peut être pourvoyeuse de myopathies toxiques. La symptomatologie varie en fonction de la quantité de substance et de la durée d'exposition.

La consommation massive d'**alcool** peut engendrer un épisode de rhabdomyolyse avec œdème des masses musculaires, myalgies et myoglobulinurie [181]. Un trouble hydroélectrique important, tel que l'hypokaliémie < 2,5 mEq peut aussi en être responsable. L'intoxication éthylique chronique est, quant à elle, responsable d'une atteinte myopathique proximale, symétrique et progressive, s'installant sur plusieurs années. Elle est fréquemment associée à une atteinte du muscle cardiaque, avec myocardiopathie dilatée [181]. Les CPK sont en général modérément augmentées. Il est important néanmoins de noter que l'atteinte musculaire est souvent multifactorielle. En effet, lors de l'addiction ou le mésusage prolongé de substances addictives, s'associent parfois une neuropathie, et souvent une malnutrition. L'analyse histologique musculaire montre une atrophie des fibres de type II, et des lésions de nécrose dans les intoxications aiguës. D'un point de vue myopathologique, l'alcool induit une résistance à certains facteurs anaboliques (notamment insuline et *insulin like growth factor 1*), et augmentent la production de régulateurs négatifs de la masse musculaire [182]. Concernant l'évolution, en deçà d'un certain seuil, l'arrêt de l'intoxication permet une lente récupération.

D'autres produits toxiques peuvent être responsables d'atteinte musculaire dont la gravité est variable. Le **gérianium** utilisé parfois en phytothérapie a été rapporté comme responsable de myopathie vacuolaire floride avec à la biopsie, une augmentation de l'activité phosphatase acide et une accumulation de lipides au sein des fibres musculaires [183]. D'autres produits, comme l'huile frelatée, responsable du **syndrome de l'huile toxique** induit des lésions des fibres musculaires, avec épaississement des fascias.

B. MYOPATHIES ENDOCRINIENNES

Les endocrinopathies sont pour la plupart des maladies de système qui peuvent toucher différents organes. L'atteinte musculaire dans le cadre des endocrinopathies, autrement appelée « myo-endocrinopathie » n'est pas rare, et probablement sous-diagnostiquée [184]. Le tableau musculaire aspécifique est d'évolution insidieuse. Chaque axe hormonal peut être en cause, au premier rang desquels, les dysthyroïdies, les troubles des hormones parathyroïdiennes et surrénaliennes. L'atteinte musculaire peut parfois précéder le diagnostic endocrinologique, et certaines atteintes évoluent, ensuite de manière indépendante. Une caractéristique non négligeable est la récupération de la fonction dans quasiment tous les cas lors de la régularisation du trouble endocrinien, ce qui souligne l'importance de ne pas méconnaître ces diagnostics.

1. Myopathies et dysthyroïdies

En cas de dysthyroïdie, l'atteinte musculaire est fréquente, pouvant toucher plus de la moitié des patients. Dans une grande majorité des cas, la symptomatologie est modérée, lentement progressive, parfois infraclinique n'aboutissant pas au diagnostic [184].

a. Myopathie dans le cadre d'une hyperthyroïdie

Même s'ils sont rarement diagnostiqués, plus de la moitié des patients semblent présenter une atteinte musculaire. Plusieurs tableaux cliniques sont possibles, avec différents phénotypes variant en fonction de la gravité de l'atteinte endocrinienne. En dehors des cas de rhabdomyolyse, les CPK sont le plus souvent normales. L'atteinte est en générale réversible suite au retour en euthyroïdie. En cas d'atteinte sévère, un traitement par bêtabloquants peut être bénéfique, par son action antagoniste des catécholamines au niveau des récepteurs β -adrénergiques, notamment les récepteurs β -1-adrénergiques qui facilitent la contraction des fibres musculaires squelettiques. La prescription de corticoïdes dans les crises thyrotoxicosiques peut, en bloquant la conversion de T4 en T3, améliorer l'atteinte musculaire. Il faut tout de même se méfier d'une myasthénie, devant la non amélioration alors que le problème endocrinien est réglé. En effet, la prévalence de cette pathologie est plus fréquente en cas d'hyperthyroïdie que dans la population générale [185].

Classiquement, cette myo-endocrinopathie survient chez les femmes ayant des goitres multinodulaires. Elle se présente sous forme d'un tableau de myopathie proximale très progressive touchant surtout les membres inférieurs, associé à une fatigue musculaire globale. Le signe du tabouret est fréquemment positif. L'amyotrophie est plutôt rare. Un tremblement fin au niveau des mains, et la présence de réflexes ostéotendineux vifs sont évocateurs du diagnostic. Des atteintes plus sévères sont possibles, comportant une faiblesse des muscles respiratoires responsable d'un essoufflement et d'une hypoventilation alvéolaire, ou encore d'une atteinte des muscles de l'œsophage avec dysphagie. En cas de thyrotoxicose, une atteinte pseudo-bulbaire peut être observée [186]. L'atteinte est généralement indolore, et les enzymes musculaires sont normales. L'EMG peut être myogène avec des potentiels polyphasiques. L'analyse anatomopathologique est très rarement faite, et est peu contributive, montrant une infiltration graisseuse associée à une atrophie des fibres de type I.

Enfin, des atteintes de type paralysie périodique pouvant être très sévère et mener le patient en réanimation ont été décrites, survenant essentiellement chez des patients hyperthyroïdiens masculins d'origine asiatique [187]. L'atteinte est corrélée à un trouble ionique de type hypokaliémie, pouvant être parfois sévère. Ce tableau peut survenir alors que le diagnostic de dysthyroïdie n'est pas posé. La prévention de la récurrence passe par la normalisation de la fonction thyroïdienne.

b. Myopathie dans le cadre d'une hypothyroïdie

Une atteinte musculaire peut survenir au décours d'une hypothyroïdie, qu'elle soit diagnostiquée ou non. Ce tableau peut aussi apparaître secondairement au traitement d'une hyperthyroïdie. Il s'agit le plus souvent d'un tableau progressif alliant une fatigue musculaire globale, un déficit proximal, des myalgies, des crampes à l'effort.

Il est décrit une lenteur de contraction et de décontraction lors de la percussion des masses musculaire au marteau réflexe. Contrairement à ce qui est retrouvé dans les atteintes musculaires associées à l'hyperthyroïdie, les enzymes musculaires sont fréquemment augmentées. L'ensemble des symptômes s'amende avec la normalisation de la fonction thyroïdienne.

Il est à noter qu'en cas d'hypothyroïdie auto-immune (maladie de Hashimoto), une association avec une MII est possible, s'intégrant alors dans des tableaux associant plusieurs atteintes dysimmunitaires [188, 189].

Le syndrome de Hoffman, aussi nommé syndrome de Kocher-Debré-Semelaigne correspond à la survenue classiquement dans l'enfance (mais parfois chez l'adulte) d'une hypothyroïdie associée à une pseudo-hypertrophie musculaire prédominant aux membres inférieurs. Les patients présentent alors un phénotype pseudo-athlétique. Ceci est à différencier de l'hypertrophie des mollets isolée, retrouvée dans les myopathies héréditaires type maladie de Duchenne. La biopsie musculaire montre des fibres de tailles normales avec parfois en leurs sein une accumulation d'acide hyaluronique et de glycogène [184]. La supplémentation en hormones thyroïdiennes permet la régression progressive de l'atteinte musculaire. Des cas de rhabdomyolyse au décours de ce syndrome ont aussi été rapportés [190].

2. Myopathie au cours des endocrinopathies surrénaliennes

L'atteinte musculaire est très fréquente en cas de **syndrome de Cushing**, avec d'importantes similitudes avec la myopathie cortisonique. Son expression est celle d'une myopathie proximale prédominant sur la racine pelvienne ainsi que les quadriceps, et est parfois associée à des myalgies. Il existe une amyotrophie sévère d'installation rapide qui n'est pas strictement corrélée au déficit musculaire. Dans les formes sévères de l'endocrinopathie, il peut s'y associer une atteinte des muscles respiratoires. Les CPK sont normales dans la majorité des cas décrits. L'atteinte est réversible avec le traitement de la pathologie endocrinologique, mais la récupération de la fonction et de la masse musculaire est longue.

En cas d'**insuffisance surrénalienne**, la moitié des patients présentent une faiblesse musculaire généralisée, avec des crampes, et parfois des contractures musculaires. Ces anomalies de la contraction musculaire peuvent être en lien avec l'hyperkaliémie. L'EMG et les CPK sont normaux. La biopsie musculaire n'est pas nécessaire, et apparaît normale en dehors d'une diminution du marquage du glycogène dans les fibres. Un traitement de l'hyperkaliémie, ainsi que la mise en place rapide d'une hormonothérapie substitutive permet la disparition des symptômes musculaires.

Il est à noter qu'une hypoplasie des surrénales a par ailleurs été décrite dans certains cas de myopathie de Duchenne, et de mitochondriopathie de type syndrome de Kearns-Sayre [191, 192].

3. Myopathies secondaires à une anomalie du métabolisme calcique et la vitamine D

a. Atteinte musculaire et hyperparathyroïdie

Indépendamment du caractère primaire ou secondaire, des symptômes de type faiblesse musculaire peuvent être observés en cas d'hyperparathyroïdie. Cette atteinte est retrouvée chez environ 15 % des patients, et se présente comme une faiblesse musculaire généralisée et un déficit proximal et symétrique des membres inférieurs. Il existe une raideur musculaire sans réelle myotonie, parfois associée à des douleurs pelviennes. Le tableau myopathique peut être le mode de révélation de la maladie, responsable d'une errance diagnostique plus ou moins longue. Il existe des signes associés qui aideront le clinicien (douleurs et déminéralisation osseuses, lithiase urinaire) ainsi que des anomalies du bilan phosphocalcique, qui permettront de rattacher une atteinte musculaire en apparence isolée à l'hyperparathyroïdie. Il existe une corrélation entre fatigue musculaire et taux de parathormone circulant. Les CPK sont, quant à elles, en général normales [193].

b. Atteinte musculaire et hypoparathyroïdie

En lien avec l'hypocalcémie et d'hypomagnésémie parfois sévère, des tableaux de tétanie peuvent être retrouvés chez les patients ayant une hypoparathyroïdie.

c. Atteinte musculaire et ostéomalacie

L'ostéomalacie et la carence en vitamine D peuvent être associées à une faiblesse musculaire proximale parfois douloureuse, non amyotrophisante [194]. Les CPK sont normales. Ce tableau aspécifique peut mener au diagnostic erroné de fibromyalgie. La présence de signes osseux tel que la déminéralisation osseuse, les fractures sans traumatismes, ainsi que des anomalies du bilan phosphocalcique et une diminution du taux de vitamine D permettent le diagnostic. Le traitement de la carence en vitamine D permet en général de corriger les troubles musculaires.

4. Affections musculaires et pathologies myopathiques

a. Axe hormone de croissance

L'hormone de croissance (GH) entraîne la sécrétion d'IGF-1 qui est une hormone anabolisante. De fait, lors de l'acromégalie, il est fréquemment observé un tableau myopathique avec faiblesse musculaire diffuse, possiblement associée à une hypertrophie musculaire. Les CPK sont normales ou modérément augmentées.

À l'inverse, lors de la carence en GH, on retrouve une fonte musculaire avec infiltration graisseuse des massifs musculaires et des anomalies du bilan lipidique [184].

b. Diabète et atteinte musculaire

La survenue d'un tableau myopathique chez les patients présentant un diabète, d'autant plus chez les patients

jeunes, doit faire rechercher avant tout des éléments en faveur d'une mitochondriopathie (surdité, ptosis, épilepsie, neuropathie sensitive ataxiante, rétinite pigmentaire, etc.) chez le patient et chez ses ascendants.

En dehors de ce cadre pathologique bien particulier, le diabète peut être associé à une atteinte musculaire dont l'expression est variable. En effet, une amyotrophie secondaire à la neuropathie périphérique peut être retrouvée. La résistance à l'insuline, présente aussi au niveau des fibres musculaires peut se compliquer de sarcopénie. Enfin, des épisodes de myonécrose par infarctus du muscle ont été rapportés [195]. Il n'y a en général pas d'anomalies des CPK. Ces atteintes musculaires surviennent essentiellement chez des patients dont la pathologie endocrinienne n'est pas contrôlée [196].

III. DIAGNOSTICS DIFFÉRENTIELS : LES MYOPATHIES HÉRÉDITAIRES

Les myopathies héréditaires peuvent parfois présenter des tableaux cliniques proches des myopathies acquises, et doivent être éliminées lors du bilan diagnostique clinique et paraclinique.

A. DÉFICIT MUSCULAIRE PROXIMAL LENTEMENT PROGRESSIF HÉRÉDITAIRE

Certains éléments sont cruciaux pour la démarche diagnostique, notamment, le mode de transmission (analysable grâce à la réalisation d'un arbre généalogique au décours de la consultation), la répartition du déficit (symétrique ou non, proximale ou distale), la présence d'une myotonie (anomalie du tonus musculaire se caractérisant par une décontraction lente et difficile du muscle à la suite d'une contraction volontaire), la survenue de rétractions ainsi que leurs distributions, et la présence d'une cardiopathie.

L'analyse anatomopathologique révèle en général une formule dystrophique, et possiblement des anomalies d'expression des protéines (variable en fonction du type de myopathie). L'étude génétique permettra le plus souvent le diagnostic.

Ci-dessous sont présentés rapidement les myopathies héréditaires se présentant comme un déficit musculaire proximal lentement progressif que l'on peut rencontrer dans nos consultations. Pour simplifier, elles sont classées en fonction de leurs modes de transmission.

1. Autosomique dominante

a. Myopathie facio-scapulo-humérale

La FSH est une myopathie héréditaire relativement fréquente (prévalence 1/20 000) [197]. Les symptômes débutent après 10 ans, associant une atteinte musculaire asymétrique et sélective incluant les muscles de la face (orbiculaire des lèvres, masséter), de la ceinture scapulaire

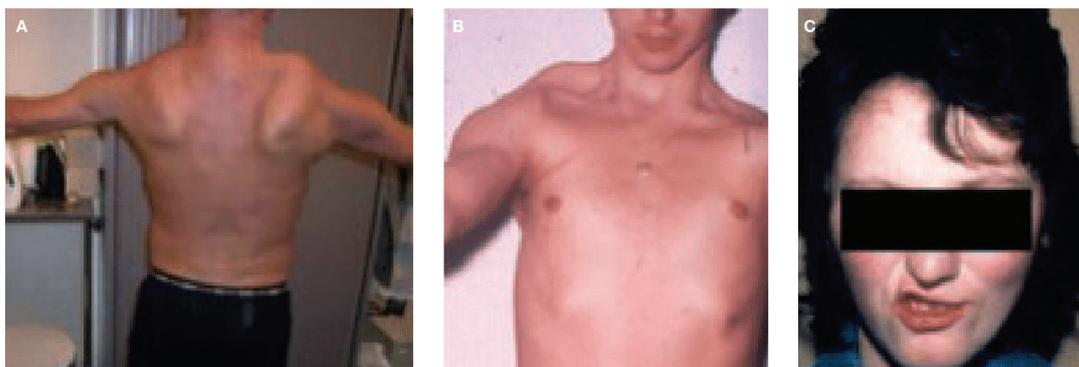


Figure 11 – Myopathie facio-scapulo-humérale. A. Décollement des scapulas. B. Atteinte asymétrique des trapèzes. C. Atteinte asymétrique des orbiculaires de bouche.

(fixateur des scapulas [omoplates], avec le signe clinique *scapula alata* (Figure 11), sterno-cléido-mastoïdiens, biceps brachiaux et long supinateurs). La confirmation du diagnostic est génétique, avec la présence de mutation FSHD1 ou FSHD2.

b. Dystrophie myotonique de Steinert

La maladie de Steinert est la plus fréquente des dystrophies myotoniques, avec une prévalence à 1/20 000 [198]. La maladie débute en général entre 20 et 30 ans, et associe un déficit moteur distal avec myotonie, un faciès évocateur (ptosis, diplégie faciale, atrophie massétérine, temporale (Figure 12) et des atteintes systémiques (cataracte, troubles de conduction et du rythme cardiaque, diabète). La confirmation est génétique avec la mise en évidence d'une expansion de triplet CTG.



Figure 12 – Dystrophie myotonique de Steinert. A. Déficit distal et atrophiante. B. Calvitie, ptosis, atrophie temporale et massétérine. C. Myotonie.

2. Récessive liée à l'X

Myopathie de Becker

La maladie est liée à une anomalie de la dystrophine et a pour pendant pédiatrique sévère la myopathie de Duchenne. Dans la maladie de Becker, les symptômes sont plus tardifs (perte de la marche dans la troisième décennie), avec une atteinte pelvi-fémorale, sans atteinte faciale, une hypertrophie des mollets, une macroglossie et une cardiomyopathie dilatée. La confirmation du diagnostic est histologique (déficit en dystrophine) et génétique (gène de la dystrophine).

3. Autosomique récessive

Dystrophies des ceintures

Il s'agit d'un groupe hétérogène ayant en commun l'absence d'atteinte faciale et la transmission autosomique récessive [199].

En cas d'hypertrophie des mollets, avec une atteinte peu sélective des cuisses (phénotype Becker-Duchenne like), il faudra en priorité évoquer les **sarcoglycanopathies (LGMD2 C,D,E,F)** et les **α -dystroglycanopathies (LGMD2I)**. La confirmation du diagnostic se fera sur la biopsie musculaire avec étude en immunomarquage (anomalies de ces protéines), sur le Western Blot mettant en évidence un déficit en sarcoglycane ou en α -dystroglycane, et grâce à l'étude génétique.

En cas d'atteinte purement atrophique et sélective touchant la loge postérieure de cuisse plus que le quadriceps, il conviendra d'évoquer les **calpainopathies (LGMD2A)** et les **dysferlinopathies (LGMD2B)**. Fait trompeur, cette dernière peut souvent s'accompagner d'infiltrats inflammatoires endomysiaux visibles sur la biopsie musculaire. La confirmation du diagnostic se fera sur la biopsie musculaire avec étude immunomarquage (anomalies de ces protéines), et l'étude génétique. Si cette dernière est négative, un panel de gènes « dystrophie des ceintures » peut être réalisé.

B. DÉFICIT MUSCULAIRE DISTAL LENTEMENT PROGRESSIF HÉRÉDITAIRE

Principal diagnostic différentiel de la myosite à inclusions, les myopathies distales peuvent être de transmission variable [200]. Leur tableau clinique allie une atteinte élective de la musculature distale, épargnant la face et l’oculomotricité.

Les myopathies distales récessives sont plutôt précoces, avec des symptômes apparaissant entre 15 et 30 ans. La **myopathie de Miyoshi** correspond à une atteinte des loges postérieures de la jambe. Les CPK sont souvent très élevées (10 à 50 fois la norme). La biopsie retrouve un muscle dystrophique avec un déficit complet en dysferline. L’étude génétique retrouve une mutation du gène *DYSF*. La **myopathie de Nonaka** se présente comme une atteinte progressive des loges antéro-externe de jambe, épargnant le quadriceps. Les CPK sont modérément augmentées (1 à 5 fois la norme). L’analyse histologique montre des vacuoles bordées, et l’étude génétique identifie une mutation du gène *GNE*. Les **anoctaminopathies** correspondent à des atteintes distales asymétriques avec atteinte des loges postérieures. La confirmation est génétique avec mutation du gène *ANO5*.

Les myopathies distales dominantes sont plutôt tardives et comprennent la myopathie à VCP de forme distale, la myopathie tibiale de Udd, les zaspopathies, et les myotilinoopathies. La confirmation du diagnostic se fait sur l’analyse génétique et anatomopathologique.

C. INTOLÉRANCE À L’EFFORT, FATIGABILITÉ MUSCULAIRE

La fatigabilité musculaire, l’intolérance à l’effort, parfois accompagnées d’épisode de rhabdomyolyse sont l’apanage des anomalies du métabolisme énergétique musculaire (voir le chapitre « Maladies du métabolisme : que doit savoir l’interniste ? », vol. 2). Ces anomalies se déclinent en anomalies du métabolisme du glycogène (glycogénose et pathologie de la glycolyse) [201], de la β-oxydation (myopathies lipidiques) ou du fonctionnement des mitochondries (mitochondriopathies) (Figure 13).

La démarche diagnostique comprend un interrogatoire complet avec recherche d’antécédents personnels et familiaux (intolérance à l’effort avec myalgies, essoufflement, épilepsie, diabète, surdité, rétinite pigmentaire, cardiopathie, ataxie cérébelleuse, etc.), ainsi que la recherche d’épisodes de rhabdomyolyse (urines couleur porto, survenant habituellement après un effort important ou un stress [jeûne, sepsis]). La topographie de l’atteinte devra être définie (éliminer un syndrome des loges pouvant évoluer de manière chronique en cas d’atteinte localisée). Le bilan paraclinique devra être orienté par la clinique. La mesure de la glycémie, les dosages de la carnitinémie et des acylcarnitines plasmatiques, la chromatographie des acides organiques urinaires, la réalisation d’une épreuve d’effort avec dosage lactatémie et ammoniémie, la biopsie musculaire (permettant

d’identifier des accumulations du glycogène ou de lipides dans les fibres musculaires), voir dans certains cas, la réalisation de biologie moléculaire ciblée (maladie de McArdle) sont les outils disponibles pour le clinicien.

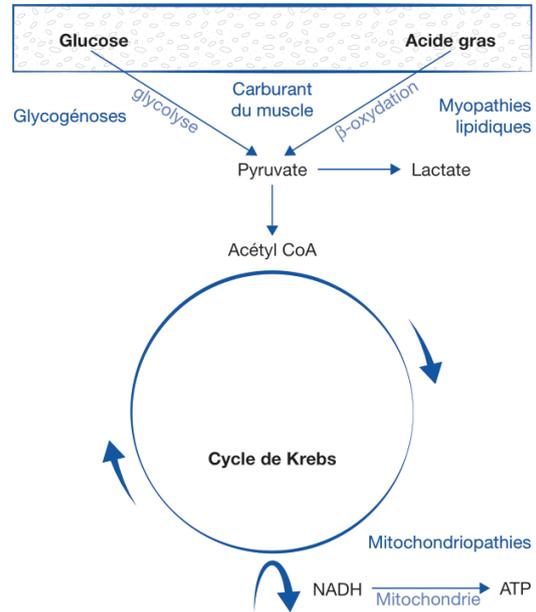


Figure 13 – Anomalies du métabolisme énergétique musculaire.

Chaque étape des voies métaboliques peut être altérée, expliquant le nombre important de pathologies, parmi lesquelles, quelques maladies nécessitent d’être connues par l’interniste.

1. Glycogénoses

La **maladie de McArdle (glycogénose type V)** correspond à un déficit en myophosphorylase, de transmission autosomique récessive [201]. La maladie survient en général dans l’enfance, mais des formes myopathiques progressives (chez les plus de 30 ans, avec atrophie musculaire), ou du sujet âgé (> 60 ans), ainsi que des formes latentes (compatibles avec la pratique du sport intensif) sont possibles. La symptomatologie allie une fatigabilité musculaire avec intolérance à l’exercice avec un phénomène de second souffle, crampes, myalgies, et parfois épisodes de myoglobinurie pouvant se compliquer d’insuffisance rénale aiguë. L’association à une goutte (hyperuricémie) est possible. Des « crises musculaires » surviennent au décours d’efforts inhabituels, parfois favorisées par certains cofacteurs (froid, alcool, stress). Les symptômes disparaissent entre les crises. Les CPK peuvent être élevées. Lors de l’épreuve d’effort (*Grip-test*), on note l’absence d’élévation des lactates, associée à une hyperammoniémie. L’analyse histologique, réalisée à distance d’un épisode de rhabdomyolyse, met en

évidence une atrophie des fibres de type 2, la présence de vacuoles sous-sarcolemmiques de glycogène et un déficit de l'activité myophosphorylase.

La **maladie de Tarui (glycogénose type VII)**, correspondant à un déficit en phosphofructokinase, est extrêmement rare. Elle associe une intolérance à l'exercice, des crampes, des épisodes de myoglobulinurie, l'absence de phénomène de second souffle, et une hémolyse chronique (l'enzyme est présente dans le muscle, dans le foie et dans les érythrocytes). Une hyperuricémie n'est pas rare. La symptomatologie est aggravée après charge de glucose. Le test d'hyperlactacidémie provoqué montre une courbe plate. L'analyse anatomopathologique montre une augmentation de glycogène dans les fibres musculaires. Le diagnostic est confirmé par l'analyse génétique avec des mutations des gènes *PFK M*, *L* ou *B*.

La **maladie de Pompe (glycogénose de type II)** correspond à une myopathie autosomique récessive avec déficit en maltase acide (α -glucosidase acide ou GAA) [202]. La forme adulte, due à un déficit partiel de l'enzyme, débute au cours de la deuxième ou troisième décennie, et prend la forme d'une myopathie lentement progressive touchant préférentiellement la ceinture pelvienne. Les patients présentent une marche dandinante parfois associée à une hyperlordose (atteinte de la musculature axiale), et une macroglossie. Des troubles respiratoires par atteinte des muscles diaphragmatiques sont possibles. Des atteintes cardiaques sous forme d'un allongement de l'intervalle PR et de dysfonction systolique ventriculaire gauche sont plus rarement rapportées (< 10%). La glycémie est normale, de même que les épreuves dynamiques (glucagon). L'épreuve d'effort montre une intégrité de la glycogénolyse. Les CPK varient de 2 à 10 fois la norme (< 1 000 UI/L). L'IRM peut mettre en évidence des anomalies des cuisses, de la ceinture scapulaire ou encore de la langue. L'analyse histologique montre de nombreuses vacuoles de glycogène dans les fibres, mais peut être normale. Le dosage sanguin ou sur papier buvard de l'enzyme GAA permet le diagnostic et l'évaluation d'une potentielle activité enzymatique résiduelle. L'analyse génétique retrouve des mutations du gène GAA. Il s'agit d'une des rares myopathies héréditaires pour laquelle un traitement est disponible (enzymothérapie substitutive).

Le **déficit en enzyme branchante (glycogénose de type III)** est une myopathie autosomique récessive liée à une mutation du gène AGL. Elle peut se présenter chez l'adulte comme l'association d'une faiblesse musculaire et d'une hépatomégalie. Le diagnostic se fait sur le dosage de l'activité enzymatique sanguine. Un traitement par régime riche en protéines ou nutrition entérale nocturne peut être bénéfique.

2. Myopathies lipidiques

Les premiers symptômes des myopathies lipidiques surviennent le plus souvent dans l'enfance, avec des myalgies, des épisodes de myoglobulinurie après des efforts intenses, parfois favorisés par d'autres facteurs (froid, fièvre, jeûne, stress). Les enzymes musculaires sont normales au repos,

de même que le *Grip-test* qui est normal. Les outils utiles pour le diagnostic sont le profil sanguin des acylcarnitines (réalisé après 10 à 12 heures de jeûne) et le dosage des acides organiques urinaires. La biopsie musculaire montre fréquemment une surcharge lipidique sur la coloration noir soudan.

Le **déficit en carnitine** peut être primaire ou secondaire [202]. L'atteinte primaire correspond à un déficit en OCTN2 (transporteur permettant à la carnitine de pénétrer dans la mitochondrie), les atteintes secondaires étant en lien avec diverses pathologies (insuffisance rénale chronique hémodialysée, cirrhose avec cachexie, insuffisance surrénalienne, myxœdème, hypopituitarisme, autres myopathies chroniques sévères et notamment les mitochondriopathies, grossesse ou encore traitement prolongé par valproate). Les tableaux cliniques sont sensiblement les mêmes, débutant chez l'adolescent ou l'adulte jeune, et alliant une fatigabilité musculaire progressive avec faiblesse des ceintures et des hypoglycémies pouvant être sévères. L'atteinte des muscles de la face est fréquente. Des atteintes cardiaques et respiratoires sont possibles. Les CPK sont augmentées, la biopsie musculaire retrouve de nombreuses vacuoles lipidiques dans les fibres de type I. La carnitine plasmatique peut être abaissée, il n'y a pas, par contre, d'anomalies du profil des acylcarnitines. Le traitement consiste en une administration de carnitine *per os*.

Le **déficit en carnitine palmitoyltransférase II (CPT)** correspond à une anomalie de la β -oxydation dans la mitochondrie [204]. Cliniquement, il existe des myalgies per-effort nécessitant l'arrêt de l'exercice, associées à des épisodes de myoglobulinurie dans les 24 heures après l'effort. Les CPK sont normales ou augmentées, la lactacidémie est normale à l'effort. L'analyse du profil des acylcarnitines montre une augmentation des acides gras à longue chaîne. Le diagnostic est confirmé par une anomalie de l'activité enzymatique de CPT2 dans les lymphocytes circulants, les muscles ou les fibroblastes en culture.

3. Myopathies mitochondriales

Les myopathies mitochondriales sont la conséquence de dysfonctionnement de la chaîne respiratoire mitochondriale [205]. Elles peuvent être héréditaires (mutation de l'ADN mitochondrial, ou mutation de l'ADN nucléaire) ou acquises (secondaire à certain traitement comme l'AZT, cf. myopathies toxiques). Tous les modes de transmission sont possibles (sporadique, hérédité mitochondriale avec transmission maternelle, autosomique dominant ou récessif, lié à l'X). La maladie peut débiter à tous les âges de la vie. Cliniquement, il s'agit d'atteinte multisystémique avec une atteinte musculaire (hypotonie, intolérance à l'effort, déficit myopathique proximal), des atteintes du système nerveux central (encéphalopathie, épilepsie myoclonique, pseudo-AVC, ataxie cérébelleuse) notamment au décours des syndromes MELAS (*Mitochondrial Myopathy Encephalopathy Lactic Acidosis and Stroke-like episodes*) et MERRF (*Myoclonus Epilepsy associated with Ragged Red Fibers*) ou périphérique (neuropathie sensitive ataxiante), des atteintes ophtalmologiques (rétinite pigmentaire,

ophtalmoplégie, ptosis, neuropathie optique), ORL (surdité de perception), endocrinologique (diabète), cardiologique (cardiomyopathie avec trouble de conduction), néphrologique (glycosurie, aminoacidurie), hématologique (cytopénies), digestive (insuffisance hépatocellulaire, insuffisance pancréatique exocrine, dysphagie, atrophie villositaire) [205]. D'un point de vue paraclinique, une hyperlactatémie est retrouvée à l'état basal (jusqu'à 10 fois la normale). La biopsie musculaire met en évidence de fibres rouges déchiquetées (*ragged-red fibers*), une surcharge en glycogène, en lipides, des anomalies des complexes de la chaîne respiratoire sur l'analyse histo-enzymologique (fibres COX négatives : complexe IV, fibres SDH diminué : complexe II). Des études génétiques peuvent être réalisées sur le muscle, le sang ou les urines, recherchant des mutations de l'ADN mitochondrial et nucléaire.

Les tableaux cliniques florides rendent les diagnostics des myopathies mitochondriales complexes. Trois éléments peuvent alerter le clinicien : l'atteinte oculomotrice, la symptomatologie multisystémique et le climat métabolique (intolérance à l'effort, fatigabilité musculaire).

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During this synthesis work, FM was identified as a poorly defined subgroup. This led us to consider the need for further investigation about this peculiar entity. New insights about clinical, investigational and pathological characteristics are presented in the results section.

1.2. IIM pathogenesis

Despite important efforts made in the last decades, the understanding of IIM etiopathogenesis remains partial, combining adaptative and innate immunity dysfunction, genetic background, and environmental factors (Ceribelli et al., 2016).

Considering the occurrence of auto-antibodies and the improvement of the disease under immunosuppressive drugs, disturbances of the adaptative immune system have been extensively explored. Various cytokines play also a role in this disease, notably pro-inflammatory cytokines such as Th1 and Th17 cytokines, Interleukin (IL)-1 family, and IFN-I (Benveniste et al., 2019; Gitiaux et al., 2016; Greenberg et al., 2005). At the cellular level, T cells are implicated, either CD4 or CD8 cells. Even if seldomly observed in muscular inflammatory infiltrates, B cell involvement is suggested by the efficacy of Rituximab in some patients (a chimeric monoclonal antibody against the CD20 protein, which is primarily found on the surface of immune system B cells) (Fasano et al., 2017). Complement may also play an important role in DM pathogenesis, since capillary loss, perifascicular atrophy and inflammatory infiltrates may be related to capillary dysfunction consequent to C5b-9 (complement final membrane attack complex) deposition (Lahoria et al., 2016; Uruha et al., 2018).

1.3. IIM and IFN

1.3.1 IFN family

Interferons are highly potent cytokines that take their name from their property to interfere with viral replication in the host (Weissmann and Weber, 1986). They are widely expressed cytokines that have antiviral, antiproliferative and immunomodulatory properties. This

cytokine family is divided into three classes (type I, II, III), all sharing the ability to induce anti-viral response after binding to their cognate receptors.

Type I IFN include IFN- α (mainly produced by plasmacytoid dendritic cells) and β (mainly secreted by fibroblasts), and they are also strongly expressed by innate immune cells (Hervas-Stubbs et al., 2011). Other type I IFN subtypes are known, namely IFN- ω and IFN- τ , but remain poorly characterized because of their overlapping function with IFN- α and IFN- β , limited tissue expression, and species-to-species differences (Capobianchi et al., 2015; Pestka et al., 2004). Type I IFNs bind to a specific cell surface heterodimeric receptor (IFN-AR for IFN- α receptor) that consists of IFN-AR1 and IFN-AR2 chains. After IFN-I is bound to its receptor, the two chains recruit Janus Activated Kinase (JAK) TYK2 and JAK1. JAK activation leads to tyrosine phosphorylation of signal transducers and activators of transcription (STAT) STAT1 and STAT2 that associate with IRF9 to form the ISGF3 complex that translocates to the nucleus and activates transcription of IFN-inducible genes or IFN-stimulated genes (ISGs) (Chen et al., 2017).

Type II IFN corresponds to IFN- γ (mainly secreted by NK and T cells), the result of one gene product, typically found in natural killer and T cells (Vilcek, 2006). Through binding to a specific receptor composed of IFN- Γ R1 and IFN- Γ R2 subunits, JAK 1 and JAK2 are recruited and lead to polymerization of STAT1 (GAF: γ activated IFN), and its translocation to the nucleus for binding to γ activated sequences (GAS) and subsequent gene activation.

Type III IFN consists in IFN- λ (equivalent of interleukins 28 and 29) which is predominantly found at the epithelial cell surface (Iversen and Paludan, 2010; Sheppard et al., 2003), and is structurally related to type I IFNs and to the IL-10 family. It has a specific receptor composed of IL-28R and IL-10R2 chains. Type III IFN signaling cascade causes activation of JAK1 and TYK2, recruitment of STAT1 which at least forms ISGF3 transcription factor.

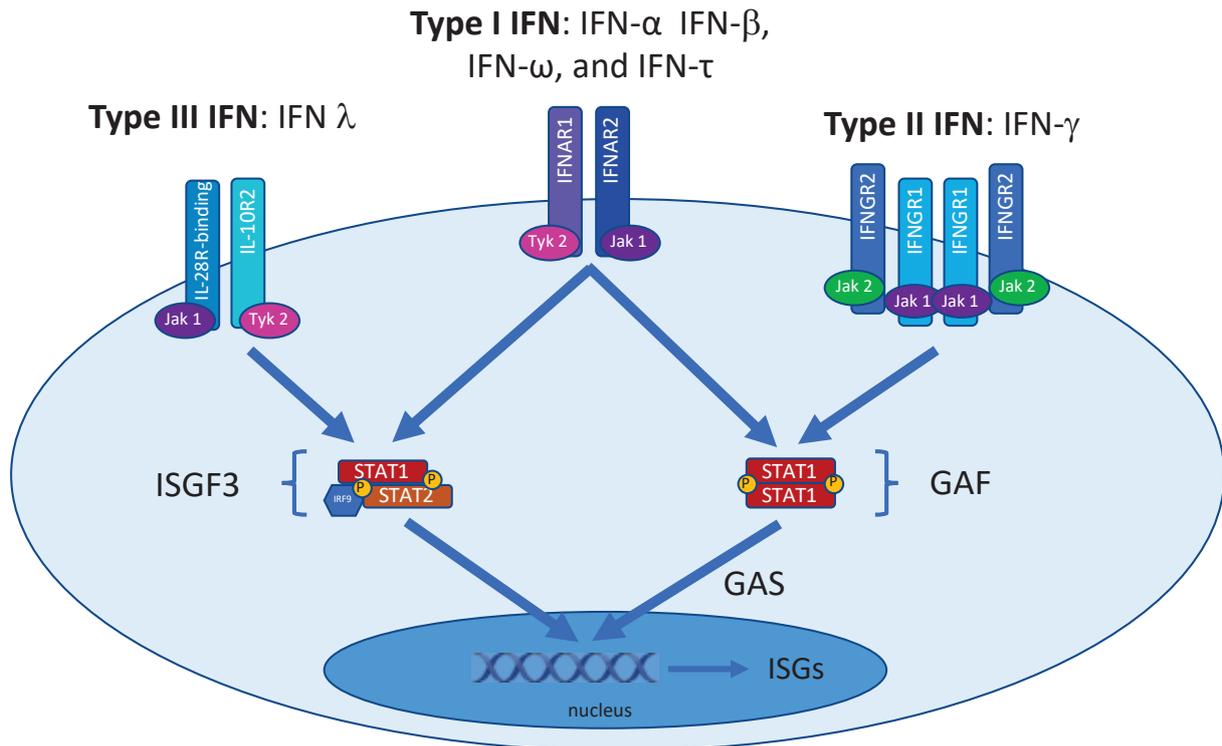


Figure 1. Interferon signaling pathways. Type I IFNs bind to a specific heterodimeric IFN Alpha Receptor (IFNAR) that consists of IFNAR1 and IFNAR2. IFN binding triggers the recruitment of Janus Activated Kinases (JAK) TYK2 and JAK1, leading to tyrosine phosphorylation of STAT1 and STAT2 that bind to IRF9 to form the ISGF3 complex, that translocates to the nucleus to stimulate the transcription of IFN-inducible genes or IFN-stimulated genes (ISGs) via binding to specific DNA binding sites (ISRE). IFN-II, which is IFN- γ , binds to the IFNGR1/IFNGR2 receptor that recruits JAK 1 and JAK2 to phosphorylate STAT1 leading to the formation of STAT1 homodimers complexes [gamma-activated factors (GAFs)], which translocate to the nucleus and targets gamma activated site (GAS) elements in the promoters of ISGs, leading to their expression [10]. IFN-III, which is IFN 1 (also known as interleukin 28 and 29), is structurally related to IFNs-I and to the IL-10 family. IFN-III binding to its receptor, composed of IL-28R-binding and IL-10R2 chains, causes the activation of JAK1 and TYK2, leading to the formation of the ISFG3 transcription factor. IFN, interferon. From Gallay et al 2019.

1.3.2 Proofs of IFN involvement in IIM

1.3.2.1 First evidence

Initially described in 1992, occurrence of IIM under IFN- α therapy, and of recovery after treatment discontinuation has led to consider IFN involvement in IIM pathogenesis (Dietrich et al., 2000; Hengstman et al., 2000; Lee et al., 2002; Shiba et al., 2014; Somani et al., 2008; Tsutsumi et al., 2002; Warabi et al., 2004). Most of these observations presented two important triggers of dysimmunity, namely IFN type I and viral infection (Hepatitis B, C, cytomegalovirus, EBV, HTLV1...).

1.3.2.2 Histological analysis

Myxovirus Resistance A (MxA) is a ubiquitous and cytoplasmic GTPase, that binds elements of the cytoskeleton (Horisberger, 1992; Richter et al., 1995). MxA is a marker of IFN-I signaling, as it is induced by IFN- α/β but not by other cytokines, notably IFN- γ . MxA is found in DM muscle fibers, regardless of the auto-antibody subgroup or life stage of disease occurrence, showing perifascicular, diffuse or scattered sarcoplasmic distribution, with 77% sensitivity, and 100% specificity (Uruha et al., 2018). In study focused on juvenile DM (jDM) on 103 patients, the sensitivity was found at 61.2%, with a strong expression of MxA in NXP2 Ab subset contrasting with the weak expression in MDA5-Ab subset supporting the hypothesis of variability of ISG expression in muscle within an IIM subgroup and related to auto-Ab occurrence (Soponkanaporn et al., 2018). This histological evidence was confirmed by immunofluorescence study of the skin (Wenzel et al., 2005). In skin of DM, strong expression of the MxA protein was detected, indicating involvement of type I IFNs. Expression of MxA was closely associated with expression of the interferon-inducible protein IP10/CXCL10 (C-X-C motif chemokine ligand 10 (CXCL10) also known as Interferon gamma-induced protein 10 (IP-10)) and the presence of lymphocytes with IP10/CXCL10 receptor CXCR3 + (a G α i protein-coupled receptor belonging to the CXC chemokine receptor family). These findings support the hypothesis of a role of IFN-I in the recruitment of inflammatory (Wenzel et al., 2006).

ISG15 is a ubiquitin modifier identified as an IFN-upregulated gene, and is overexpressed in perifascicular areas (Salajegheh et al., 2010). Positive staining was found in perifascicular

areas, in atrophic DM fibers but, interestingly, not in MDA5-Ab DM subset (Allenbach et al., 2016).

Retinoic acid inducible-gene I (RIG-I), an RNA helicase enzyme, is known as IFN-I regulatory factor, activated by innate recognition of cytosolic dsRNA. RIG-I has been identified by immunohistochemistry to be overexpressed in pathological DM muscle fibers (Suárez-Calvet et al., 2014). In a larger cohort (n=115 IIM), the same authors have studied RIG-I expression, demonstrating a positivity in 50% of DM samples, as compared with 11% in non-DM samples. The positivity was found in 32% of DM patients without perifascicular atrophy (Suárez-Calvet et al., 2017).

1.3.2.3 IFN detection in IIM

IFN may act in IIM etiopathogenesis through secreted/soluble IFN. The direct quantification of type I IFNs has been challenging, as circulating IFN soluble proteins are found at very low levels, which may reflect the high biological potency of these cytokines, most cell types expressing a type I IFN receptor.

This hypothesis was evaluated in several studies through patient IFN level analysis by ELISA (enzyme-linked immunosorbent assay) technologies. Chen et al found a correlation of serum levels of IFN- β with cutaneous lesions and occurrence of ILD in DM patients (Chen et al., 2018). Shen et al reported a significant increase of IFN- γ levels in serum of DM and PM cohorts. This was correlated with the muscular enzyme levels (creatine kinase (CK)) and occurrence of ILD (Shen et al., 2012). On a global cohort of 93 IIM patients and 36 healthy control, Liao et al found detectable level of IFN- β in blood by ELISA for 64% of untreated or minimally treated DM and 35% of all DM samples compared with 6% of other inflammatory myopathy and 6% of healthy control samples. Blood IFN β significantly correlated with high blood type 1 IFN-inducible gene expression (Liao et al., 2011).

Moreover, the usefulness of IFN ELISA has been debated, as the outcome and clinical correlation may differ according to the study considered. Indeed, some authors reported a lower IFN- α serum levels in IIM than in healthy control patients and that, independently of their treatment status (Król et al., 2011). Same authors found a negative correlation between muscular magnetic resonance imaging (MRI) intensity and IFN- α serum level (Król et al.,

2011). However, others reported high levels of IFN- α and IFN- ω , but these sera did not induce an IFN response in a bioassay of IFN activity (Liao et al., 2011).

Currently, ELISAs are considered as either insensitive or unreliable, leading to the development of proxy assays based on type I IFN signaling such as single-molecule array (Simoa) or digital ELISA technology (Berger Rentsch and Zimmer, 2011; Hua et al., 2006; Niewold et al., 2009; Rius-Rocabert et al., 2019; Seo et al., 2009). Digital ELISA technology is based on counting individual enzyme-labeled immunocomplexes of proteins captured on paramagnetic beads in Simoa (Rissin et al., 2010; Wilson et al., 2016). Direct quantification of IFN α at attomolar (femtograms per milliliter) concentrations was allowed by the combination of this technology with unique high-affinity antibodies isolated from *APSI/APECED* mutation patients, a well-known hereditary interferonopathy (Meyer et al., 2016). This technology is reported highly correlated with ISG expression in sera of patients and with IFN antiviral activity, assessed by a cytopathic protection assay (Lebon et al., 1979, 1988; Palmer et al., 2007). Using these technologies, Rodero et al found a significant increase of IFN- α level in blood in a cohort of 43 jDM in comparison to healthy donors (n=20) (Rodero et al., 2017).

Another way to evaluate IFN involvement is to use reporter cells to measure the ability of sera to cause transcription of 3 main ISGs (IFIT-1, MX-1 and PKR). In this study, IFN- α activity was significantly increased in jDM as compared with pediatric and adult healthy controls, and this was correlated with muscular enzyme increase in blood at the time of diagnosis (Niewold et al., 2009).

1.3.2.4 Transcriptomic analysis and IFN signature

At the end of the last century, studies on IIM pathogenesis tended to focus on the analysis of deeper change: the study of transcriptome. Indeed, elucidating the molecular pathways active in pathologic tissues has important implications for defining disease subsets, monitoring disease activity, and choosing therapy.

In 1995, cytokine gene expression monitoring demonstrated the up-regulation of IFN- γ in muscles of IIM patients (n=7/15) using reverse transcriptase PCR methods (Lundberg et al., 1995). These findings led to the identification in altered DM muscle of perifascicular overexpression of STAT1 (Illa et al., 1997a), which is known to be induced by IFN- γ .

Consequently, Illa et al demonstrated the overexpression of STAT1 mRNA in cultured myoblasts after a treatment with IFN- γ (Illa et al., 1997b). These studies opened the way to the consideration of transcriptomic changes in IIM pathogenesis, a new way to evaluate dysregulation of muscle and immune system in this disease.

Transcriptomic analysis has been at first conducted *in vivo* in IIM muscle, then expanded to skin, peripheral blood mononuclear cell (PBMC), and, *in vitro*, in myogenic precursor cells (MPCs) and endothelial cells (ECs) from patients (Table 3.) (Gallay et al., 2019). In these studies, IFNs appear as key elements of IIM pathogenesis. Indeed, in some studies, 21 of the top 25 most upregulated genes are known or presumed to be upregulated by IFN (Der et al 1998, Sanda et al, Indraccolo et al), including *CXCL10*, *IFIH1*, *ISG15/UBE2L6*, *C1S/R*, *IRF7*, *IDO1*, *MXB* and *CHN1* (Wong et al., 2012). Interestingly, studies demonstrated that the various subtypes of inflammatory myopathies have distinct gene expression signature (Greenberg et al., 2002, 2005; Walsh et al., 2007). Consecutively, a specific focus on IFN-induced transcripts led to the definition of "IFN signature" which represent" a further step in the understanding of the disease pathogenesis, and a new tool for diagnosis and follow-up of patients.

Table 1. Studies reporting upregulation of interferon-stimulated gene transcripts in idiopathic inflammatory myopathies

Genes induced by	IFN- α /b				IFN- γ	
	Skeletal muscle	PBMCs	Skin	MPCs/ECs	Skeletal muscle	Skin
DM	Greenberg <i>et al.</i> [13]; Greenberg <i>et al.</i> [14]; Zhou <i>et al.</i> [18]; Salajegheh <i>et al.</i> [19]; Allenbach <i>et al.</i> [29]; Rigolet <i>et al.</i> [16**]	Walsh <i>et al.</i> [15]; Baechler <i>et al.</i> [23]; Liao <i>et al.</i> [28]; Greenberg <i>et al.</i> [33]; Reed <i>et al.</i> [30]; Zhu <i>et al.</i> [24]; Huard <i>et al.</i> [31]; Zhang <i>et al.</i> [25*]	Wong <i>et al.</i> [12]		Rigolet <i>et al.</i> [16**]	Wong <i>et al.</i> [12]
JDM	Tezak <i>et al.</i> [17]; Salajegheh <i>et al.</i> [19]	O'Connor <i>et al.</i> [21]; Baechler <i>et al.</i> [23]; Reed <i>et al.</i> [30]; Huard <i>et al.</i> [31]		Gitiaux <i>et al.</i> 2018 [20*]		
IBM	Greenberg <i>et al.</i> [14]; Salajegheh <i>et al.</i> [19]; Rigolet <i>et al.</i> [16**]	Walsh <i>et al.</i> [15]			Greenberg <i>et al.</i> [13]; Raju <i>et al.</i> [26,27]; Rigolet <i>et al.</i> [16**]	
PM	Zhou <i>et al.</i> [18]; Salajegheh <i>et al.</i> [19]; Greenberg <i>et al.</i> [14]	Walsh <i>et al.</i> [15]			Greenberg <i>et al.</i> [13]	
IMNM	Rigolet <i>et al.</i> [16**]; Greenberg <i>et al.</i> [14]				Rigolet <i>et al.</i> [16**]	
ASS	Rigolet <i>et al.</i> [16**]				Rigolet <i>et al.</i> [16**]	

ASS, anti-synthetase syndrome; DM, dermatomyositis; ECs, endothelial cells; IBM, inclusion body myositis; IIMs, idiopathic inflammatory myopathies; IMNM, immunomediated necrotizing myopathies; JDM, juvenile dermatomyositis; MPCs, myogenic precursor cells; PBMCs, peripheral blood mononuclear cells; PM, polymyositis.

Table 3. Studies reporting about transcriptomic analysis, IFN signature and IFN score in IIM. (From Gallay et al 2019).

While transcriptomic analysis, IFN signature and IFN score popping up in numerous studies, no consensus are defined for the use of such tools in IIM medical care. A review has been made on IFN-signature in idiopathic inflammatory myopathies (IIMs), in order to summarize knowledge, evaluate interests and limitations of this recent tool in clinical practice and the research.

Gallay et al, IFN signature in myositis: a review. Curr Op Rheum 2019.



Interferon-signature in idiopathic inflammatory myopathies

Laure Gallay^{a,b}, Guy Mouchiroud^a, and Bénédicte Chazaud^a

Purpose of review

The present review describes the interferon (IFN)-signature currently emerging as a tool for the diagnosis of idiopathic inflammatory myopathies (IIMs), and aims at presenting the interests and limitations of this recent tool for the clinics and the research.

Recent findings

Recent in-vivo and in-vitro transcriptomic studies have evidenced the involvement of IFNs in the pathogenesis of IIMs. A correlation between the IFN-signature and the clinical severity of IIMs has been established. Moreover, studies pointed out differences in the IFN-signature regarding the IIM subgroup (dermatomyositis, polymyositis, inclusion body myositis, anti-synthetase syndrome, immuno-mediated necrotizing myopathies), raising the hypothesis of several pathogenic processes in IIMs.

Summary

IIM pathogenesis remains partially understood. IFN-signature represents one of the main recent advances in the field. IFN-signature was identified thanks to transcriptomic analyses of tissues or cells from IIM patients (muscle, skin, blood cells, muscle cells) and should allow to establish new diagnosis and better monitoring of IIM patients. It also provides a tool for investigation of IIM pathogenesis. Nevertheless, IFN-signature still requires accurate definition in order to standardize its use, notably in the clinical practice.

Keywords

idiopathic inflammatory myopathies, interferon score, interferon-signature

INTRODUCTION

Idiopathic inflammatory myopathies (IIMs) are classified as dermatomyositis, polymyositis, inclusion body myositis (IBM), anti-synthetase syndrome (ASS) and immunomediated necrotizing myopathies (IMNM) [1–4,5^{**}]. IIMs are characterized by muscle impairment and are associated with specific autoantibodies. Current medical care includes immunosuppressive drugs. Despite important efforts made during the last decades, the understanding of IIM etiopathogenesis remains fragmented, combining the involvement of adaptative and innate immunity dysfunction, of genetic background, and of environmental factors, such as virus infection or cancer [6]. Generally, elucidating specific molecular pathways involved in a disorder has important outcomes for defining disease subgroups, monitoring the disease activity and choosing therapies. In that context, interferons (IFNs) were identified as important actors in IIMs. In 1995, the analysis of cytokine expression demonstrated the up-regulation of IFN- γ in IIM muscle [7]. This finding led to identify an overexpression

of the IFN γ -induced signal transducer and activator of transcription 1 (STAT1) in the altered perifascicular area of dermatomyositis muscle [8]. This was the beginning of considering transcriptomic changes in IIM pathogenesis. IFNs are widely expressed cytokines that exhibit antiviral, antiproliferative and immunomodulatory properties. IFN family is divided into three classes, which share overlapping signaling pathways (Fig. 1) [9–11]. The present review aims at summarizing the work recently published on the IFN-signature that was found in IIMs, and to discuss the establishment of an IFN-score as a tool for diagnosis and monitoring of IIM patients.

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KEY POINTS

- ISG differential expression constitutes an IFN-signature in IIMs.
- IFN-signature (and IFN-score) correlates with the severity of the disease.
- IFN-signature (IFN-I and IFN-II) differs among IIM subgroups.
- IFN-signature needs to be standardized for a general use in clinics and for investigation of IIM pathogenesis.

leukocytes, then *in vitro* to myogenic precursor cells (MPCs) and to endothelial cells isolated from patients (Table 1). The most upregulated interferon-stimulated genes (ISGs) are varying depending on the cell type, albeit common ISGs are always upregulated, such as *OAS1*, *IFIT1*, *MxA*. These studies highlight that IFNs appear to be key effectors of IIM pathogenesis [12]. Interestingly, the different subtypes of IIMs exhibit distinct gene expression signatures [13–15,16**]. Consecutively, a focus on the analysis of IFN-induced transcripts led to the establishment of a so-called IFN-signature, which represents a further advance in the understanding of the disease, as well as a useful tool for diagnosis and monitoring IIM patients.

TRANSCRIPTOMIC EVIDENCE OF INTERFERON INVOLVEMENT IN IDIOPATHIC INFLAMMATORY MYOPATHIES

Transcriptomic analysis was first conducted *in vivo* using IIM muscle, was expanded to skin, circulating

Transcriptomic analysis of skeletal muscle

The upregulation of ISG transcripts correlated with the over expression of the corresponding proteins in

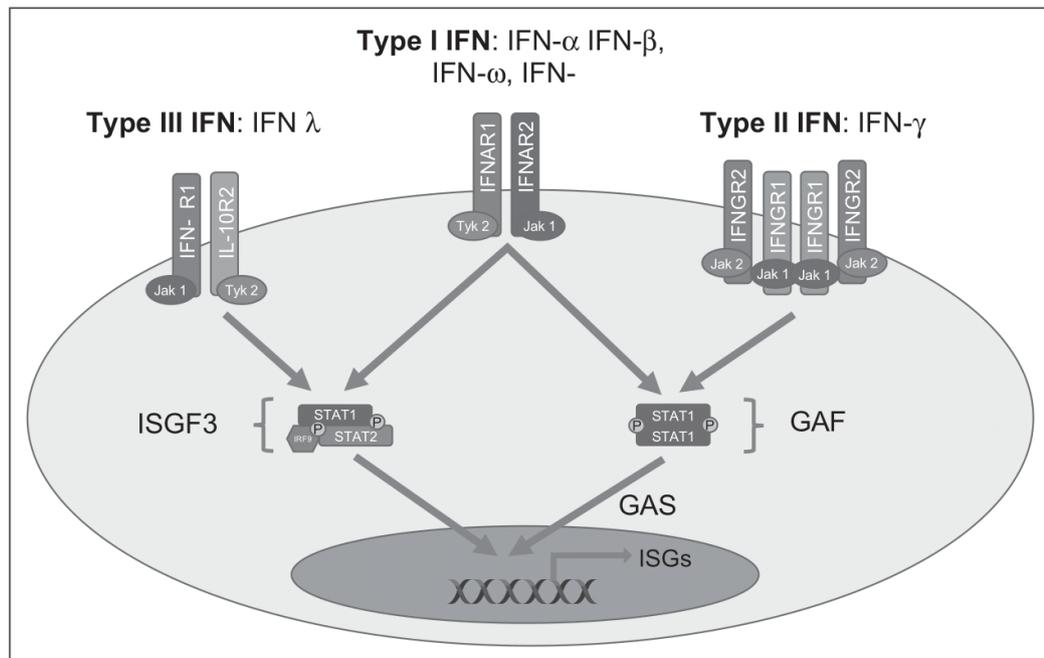


FIGURE 1. Interferon signaling pathways. Type I IFN-I include IFN- α , IFN- β , IFN- ω , and IFN- τ , the two latter remaining poorly characterized because of their overlapping functions with IFN- α and IFN- β , their limited tissue expression, and species-to-species differences [9]. Type I IFNs bind to a specific heterodimeric IFN Alpha Receptor (IFNAR) that consists of IFNAR1 and IFNAR2. IFN binding triggers the recruitment of Janus Activated Kinases (JAK) TYK2 and JAK1, leading to tyrosine phosphorylation of STAT1 and STAT2 that bind to IRF9 to form the ISGF3 complex, that translocates to the nucleus to stimulate the transcription of IFN-inducible genes or IFN-stimulated genes (ISGs) via binding to specific DNA binding sites (ISRE). IFN-II, which is IFN- γ , binds to the IFNGR1/IFNGR2 receptor that recruits JAK 1 and JAK2 to phosphorylate STAT1 leading to the formation of STAT1 homodimers complexes [gamma-activated factors (GAFs)], which translocate to the nucleus and targets gamma activated site (GAS) elements in the promoters of ISGs, leading to their expression [10]. IFN-III, which is IFN λ (also known as interleukin 28 and 29), is structurally related to IFNs-I and to the IL-10 family. IFN-III binding to its receptor, composed of IL-28R-binding and IL-10R2 chains, causes the activation of JAK1 and TYK2, leading to the formation of the ISGF3 transcription factor [11]. IFN, interferon.

Table 1. Studies reporting upregulation of interferon-stimulated gene transcripts in idiopathic inflammatory myopathies

Genes induced by	IFN- α /b				IFN- γ	
	Skeletal muscle	PBMCs	Skin	MPCs/ECs	Skeletal muscle	Skin
DM	Greenberg <i>et al.</i> [13]; Greenberg <i>et al.</i> [14]; Zhou <i>et al.</i> [18]; Salajegheh <i>et al.</i> [19]; Allenbach <i>et al.</i> [29]; Rigolet <i>et al.</i> [16**]	Walsh <i>et al.</i> [15]; Baechler <i>et al.</i> [23]; Liao <i>et al.</i> [28]; Greenberg <i>et al.</i> [33]; Reed <i>et al.</i> [30]; Zhu <i>et al.</i> [24]; Huard <i>et al.</i> [31]; Zhang <i>et al.</i> [25*]	Wong <i>et al.</i> [12]		Rigolet <i>et al.</i> [16**]	Wong <i>et al.</i> [12]
JDM	Tezak <i>et al.</i> [17]; Salajegheh <i>et al.</i> [19]	O'Connor <i>et al.</i> [21]; Baechler <i>et al.</i> [23]; Reed <i>et al.</i> [30]; Huard <i>et al.</i> [31]		Gitiaux <i>et al.</i> 2018 [20*]		
IBM	Greenberg <i>et al.</i> [14]; Salajegheh <i>et al.</i> [19]; Rigolet <i>et al.</i> [16**]	Walsh <i>et al.</i> [15]			Greenberg <i>et al.</i> [13]; Raju <i>et al.</i> [26,27]; Rigolet <i>et al.</i> [16**]	
PM	Zhou <i>et al.</i> [18]; Salajegheh <i>et al.</i> [19]; Greenberg <i>et al.</i> [14]	Walsh <i>et al.</i> [15]			Greenberg <i>et al.</i> [13]	
IMNM	Rigolet <i>et al.</i> [16**]; Greenberg <i>et al.</i> [14]				Rigolet <i>et al.</i> [16**]	
ASS	Rigolet <i>et al.</i> [16**]				Rigolet <i>et al.</i> [16**]	

ASS, anti-synthetase syndrome; DM, dermatomyositis; ECs, endothelial cells; IBM, inclusion body myositis; IIMs, idiopathic inflammatory myopathies; IMNM, immunomediated necrotizing myopathies; JDM, juvenile dermatomyositis; MPCs, myogenic precursor cells; PBMCs, peripheral blood mononuclear cells; PM, polymyositis.

altered myofibers and in capillaries of dermatomyositis muscle assessed by immunohistochemistry [14]. These results led to the hypothesis of a production of IFN-I-inducible transcripts and proteins by the myofibers, and suggested that the alteration of myofibers may be directly linked to the IFN-I downstream activation. Such an upregulation was confirmed in a transcriptomic study on juvenile dermatomyositis (JDM) muscles [17]. Using gene expression microarrays, Greenberg *et al.* [13] reported a molecular signature that distinguished IIMs from nemaline myopathies, Duchenne muscular dystrophy, congenital myopathy, and normal muscle. Focusing on dermatomyositis, they reported that 84% of the top 25 upregulated genes were ISGs. These results were confirmed on six polymyositis and four dermatomyositis muscles [18]. In a cohort of various IIM subgroups, it was found that ISG transcripts were more abundant in dermatomyositis than in other IIM muscles [14], leading to define the specific status of acquired interferonopathy for dermatomyositis. In a large IIM cohort, the expression of the ISG15-conjugation pathway (*ISG15*, *HERC5*, *USP18*) was found upregulated specifically in dermatomyositis muscles [19]. Recently, a study established an IFN- γ score to classify IIMs, defining specific IFN- γ score for IBM and ASS subgroups [16**]. *In vitro*, transcriptomic analysis of MPCs freshly isolated from JDM muscle

showed that 18 of the 30 most upregulated genes were ISGs [20*]. Similarly, endothelial cells isolated from the same JDM patients showed ISGs as 10 amongst the 30 most upregulated genes [20*].

Transcriptomic analysis of circulating leukocytes

Activation of the IFN-I pathway has also been identified in circulating leukocytes or peripheral blood mononuclear cells (PBMCs) from IIM patients. It was first assessed by the upregulation of *MxA* mRNA expression in PBMCs of JDM patients [21]. By comparing the IFN-signature observed in systemic lupus erythematosus (SLE), Baechler *et al.* [22] showed that blood cells of dermatomyositis patients present an IFN-signature. The cluster of ISGs upregulated in dermatomyositis patients was composed of 93 genes, amongst which 43 were also found in the SLE IFN-signature. Interestingly, a similar clustering was observed in both adult dermatomyositis and JDM PBMCs [23]. It is to mention that whereas most of the patients exhibited an upregulation of ISGs, 2 out of 12 did not present that molecular signature [23]. As observed in skeletal muscle, differential upregulation of ISGs in PBMCs was shown to distinguish IIM subgroups [24]. In a recent study, Zhang *et al.* [25*], showed an up-regulation of 4 ISGs (*IRF7*, *STAT1*, *ISG15* and *Mx1*) in PBMCs isolated from anti-

MDA5 antibody associated dermatomyositis, as compared with ASS and seronegative dermatomyositis patients opening the question of the heterogeneity of IFN-signature within IIM subgroups, here dermatomyositis.

Transcriptomic analysis of skin

Whole genome analysis using oligonucleotide arrays identified 946 genes differentially regulated in dermatomyositis versus normal skin [12]. Two third of those genes were up-regulated, including an important cluster of ISGs. The authors proposed a 'DM signature', which was confirmed using another microarray platform [12]. Moreover, comparative analysis of multiple inflammatory skin diseases indicates that the IFN-signature appears to be specific of dermatomyositis [12].

INTERFERON-SIGNATURE IN IDIOPATHIC INFLAMMATORY MYOPATHIES

IFN appears a key feature in the pathogenesis of IIMs, especially in dermatomyositis and JDM. IFN-signature was established by multiple techniques from various tissues. Two main approaches were used to establish the IFN-signature in IIMs. One is to identify the gene ontology of differentially expressed genes after transcriptomic analysis of the tissue samples. The second strategy was to analyze the expression of predetermined ISGs by RTqPCR on tissue samples from IIM patients, which confirmation was sometimes made on normal cells treated *in vitro* by recombinant IFNs [12–15,16²²,17–19,20²¹,23,24,25²⁶,26–31]. The definition of ISGs represents a key element for the analysis, and was used to establish a so-called IFN-score in eight IIM studies (Table 2).

Interferon score

IFN-score (Table 2) was initially established in SLE, using PBMCs, and was based on the expression levels of genes included in the IFN cluster [22]. Thereafter, eight studies proposed to score the IFN-signature in IIMs [12,16²²,23,29–33] and two in genetic interferonopathies [34,35] using various strategies. Most of the studies established a median fold change on ISG expression in IIM versus healthy control samples on a number of ISGs ranging from 6 to 21. The cut-off fold change to establish the score ranged from 2.466 to 4 [12,16²²,29,31–33]. Two studies summed the normalized expression of ISGs in IIMs versus healthy controls [23,30]. Despite a high heterogeneity in the number of genes that were analyzed (3–43), in the tissue origin (PBMC, skin,

skeletal muscle) and in the patient subgroup (dermatomyositis, IIM, IMNM, ASS, IBM), all studies have shown that the IFN-score discriminated IIM patients from healthy controls and in some cases, the severity of the disease. Therefore, the IFN-score seems a highly promising tool to investigate IIMs, although it requires standardization for a broader use.

Type I interferon versus type II interferon expression and activity

Type I and II IFNs both induce the expression of a largely overlapping group of molecules whose relationship is highly dependent on the responding cell type and on IFN concentration on the target cell. However, a study on skin from dermatomyositis patients showed that over the 10 IFNs, IFN- β and IFN- γ both strongly correlated with the IFN-signature, whereas IFN- α did not, although it was the highest expressed in the samples [12]. This suggests an uncoupling between the expression of IFN subtypes and their biological activity [12]. Interestingly, outside the field of IIMs, an *in vitro* study using human submandibular gland epithelial cell line showed that the majority of the genes that were highly up-regulated by IFN- α were also highly up-regulated by IFN- γ , making the IFN-signature a broader marker of IFN activity [36]. Nevertheless, the authors identified two genes upregulated only by IFN- γ : *GBP1* and *GBP2* [36].

Interferon-signature: bias and limitations

The variation in the range of fold changes of ISG expression, and more generally, the heterogeneity of magnitude of IFN-signature between studies remain subjects of caution. Although patients and tissue intervariability may explain that heterogeneity, the novel IFN-signature still suffers from a lack of technical standardization.

It is widely acknowledged that heterogeneity exists among IIM patients, regarding clinical and pathological phenotypes, clinical course and response to therapy. Variability may also be because of the timing of sample collection, as sample characteristics likely evolve with the course of the disease. Another limitation is the variability of the IFN-signature when patients receive a treatment. Indeed, it was shown *in vivo* [30] and *in vitro* [37] on SLE PBMCs that high doses of glucocorticoids, a standard treatment of disease flares, shut down the IFN-signature.

Moreover, important variations in the IFN-signature were observed between the tissues isolated from a same patient [15]. Variations may correlate

Table 2. Studies reporting interferon score in idiopathic inflammatory myopathies

Reference	Baechler et al. [23]	Greenberg et al. [33]	Reed et al. [30]	Rice et al. [34]	Wong et al. [12]	Rice et al. [35]	Allenbach et al. [29]	Huard et al. [31]	Rice et al. [32]	Rigolet et al. [16**]
Disease	SLE and IIM (DM)	IIM (DM and PM)	IIM (DM)	Genetic interferonopathies	IIM (DM), other inflammatory skin diseases	Genetic interferonopathies	IIM (DM)	IIM (DM)	Genetic interferonopathies, IIM (DM), SLE, sjIA	IIM (DM, IMNM, IBM, ASS)
Tissue	PBMC	PBMC	PBMC	PBMC	Skin	PBMC	Muscle	PBMC	PBMC	Muscle
Technique	Microarray + gene ontology	RTqPCR	RTqPCR	RTqPCR	Oligonucleotide arrays + RTqPCR	RTqPCR	RTqPCR	RTqPCR	qPCR	RTqPCR
IFN-score calculation	Sum of normalized expression values versus healthy controls	FC versus healthy controls: weak (FC < 4), moderate (4 ≤ FC < 10), high (FC > 10)	Sum of normalized expression versus healthy controls adjusted to a 100-point scale	Median FC versus healthy controls	Median expression value (versus healthy controls)	Median FC versus healthy controls Positive score 2.466	Mean RQ of the six genes	Mean FC: low less than 3, high greater than 3	Positive score 2.466	Median FC versus healthy controls
Number of ISGs	43	13	3	15	21	6	6	10	6	6
IFN-I ISGs										
IFI27		x		x		X		x	x	
IFI44L	X	x		x	x	X		x	x	
IFIT1	X	x	x	x	x	X		x	x	
ISG15		x		x	x	X		x	x	
RSAD2	X			x		X		x	x	
SIGLEC1				x		X			x	
Ly6E	X			x						
MX1	X	x		x						
USP18	X			x						
OAS1	X	x		x			x			
IFI44	X	x		x				x		
IFI6		x		x	x			x		
IFIT3	X	x		x	x					
IRF7	X		x	x						
STAT1	X			x			x			
ETV7	X									
AW474434	X									
FAM46A	X									
SAMD9	X									
SNF7DC2	X									
CBP1	X									
SP100	X									
TNFSF10	X									
EIF2AK2	X									
NM_00595	X									
SN	X									
IL1RN	X									
MX2	X				x					
IFRG28	X									
ZC3HDC1	X									

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Table 2 (Continued)

Reference	Baechler et al. [23]	Greenberg et al. [33]	Reed et al. [30]	Rice et al. [34]	Wong et al. [12]	Rice et al. [35]	Allenbach et al. [29]	Huard et al. [31]	Rice et al. [32]	Rigolet et al. [16**]
OASL	X				x					
HERC6					x					
HERC5	X	x						x		
OAS2	X									
OAS3	X	x					x			
HSXIAPAF	X									
G1P2	X		x							
G1P3	X									
IFIT2	X									
TRIM22	X									
IFIT5	X									
PLSCR1	X									
IFI16	X									
ZBP1	X									
LAMP3	X				x					
TRIM5	X									
PHF11	X									
ZCCHC2	X									
MDA5							x			
RIG-1							x			
CXCL10					x			x		
CMPK2								x		
BATF2					x					
IFIT2					x					
IFIH1					x					
SAMD9					x					
IDO1					x					
PARP14					x					
ISG20					x					
BST2					x					
EPSTI1		x			x					
IFN-II ISGs										
IFNg							x			
gBP2										x
Hla-DOB										x
Hla-DPB										x
ciita										x
Hla-Drb										x
Hla-DMB										x

ASS, anti-synthetase syndrome; DM, dermatomyositis; ECs, endothelial cells; FC, fold change; IBM, inclusion body myositis; IIMs, idiopathic inflammatory myopathies; IMNM, immunomediated necrotizing myopathies; JDM, juvenile dermatomyositis; MPCs, myogenic precursor cells; PBMCs, peripheral blood mononuclear cells; PM, polymyositis.

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with the clinical phenotypes that differentially affect skin or muscle in each dermatomyositis patient [12]. Using a large cohort, Wong *et al.* [12] observed that PBMCs from dermatomyositis and SLE

patients showed a weaker induction of ISGs than the skin samples from the same patients.

On top of patient and tissue variability, technical procedures should be carefully driven as sample

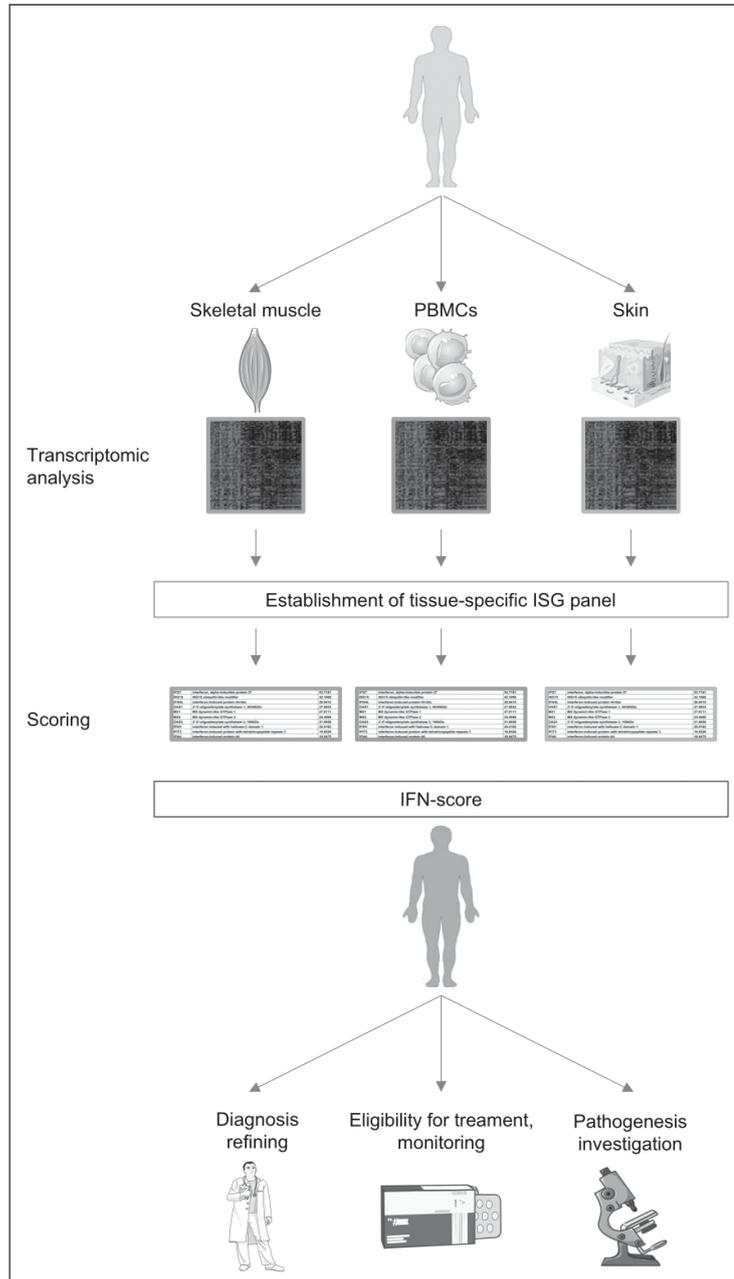


FIGURE 2. Establishment and standardization of tissue-specific IFN-score. IFN-I-signature should be standardized to establish tissue-specific IFN-score that will help to better diagnose the patients, monitor the evolution of the disease and select responding patients for future therapeutics. IFN, interferon.

handling may induce changes in gene expression (as shown in PBMCs, [22]) and is currently not standardized. For instance, blood sample handling was described to range from 2 to 72 h before gene expression analysis was performed [32,34,35]. Finally, the variety of probes that have been used so far also appears as so many causes of variability.

APPLICATION TO THE CLINICS

A plethora of studies showed that the IFN-signature correlates with the disease severity [21,23,30,31,33]. Moreover, the IFN-score established from dermatomyositis-derived PBMCs correlated with the disease activity score based on muscle strength testing, muscle enzyme elevation, skin disorder and patient report of functional assessment [23]. Similarly, transcriptomic analysis of skin from dermatomyositis patients showed that patients with inactive-skin dermatomyositis clustered with the healthy group and segregated from the active-skin dermatomyositis patients [12]. According to the studies described above, it appears that IFN-signature varies depending on the IIM subgroup, with an IFN-I-signature for dermatomyositis and JDM, and an IFN-II-signature for ASS and IBM [14,16[■],19,29–31]. As IFN-signature appears to be an easy test, specifically when made on blood sample (i.e. PBMCs), it is being developed at the hospital for refining the diagnosis of IIM patients and will help to identify whose patients are eligible for anti-IFN therapies. Moreover, the establishment of a standardized IFN-signature will provide a tool for the evaluation of IIM treatments, including anti-IFN therapies [38[■]], as it was done for SLE patients [39].

As described above, IFN expression does not correlate with IFN-signature and IFN activity. On a practical point of view, transcriptomic IFN-signature appears to be a more sensitive readout than the evaluation of the serum cytokine levels in IIMs [33,40]. Indeed, although studies reported a positive correlation between the severity of the disease and the level of IFN- β in dermatomyositis serum, and of IFN- γ , and IFN- α in JDM serum [41–43], others reported no correlation [40] or discrepancies between IFN- α and IFN- β correlation with dermatomyositis severity [31]. Moreover, the detection of IFN proteins by ELISA is hardly feasible in hospital routine, as the protein quantity ranges in atomolar concentrations, and thus requires specific technology for its detection, such as single-molecule assay (Simoa) [44], which remains currently in the research field [20^{*}].

CONCLUSION

A dozen of publications from different laboratories have shown the interest and the robustness of using

the IFN-signature in the analysis of IIMs. However, to date, there is no consensus on the list of genes to be included in the IFN-signature and the way to calculate an IFN-score, preventing the diffusion of this promising technique for a wide use. It is likely that standardization of the IFN-signature and/or IFN-score requires: the definition of a list of ISGs to be analyzed, the establishment of procedures in the handling of samples to limit variability among laboratories/hospitals, the definition of the techniques for the quantification of ISG transcripts, that will likely move towards RNAseq in the future (for which standardization will be also required). Moreover, discrepancies of the IFN-signature between tissues from the same patient suggest different signaling pathways, in accordance with the respective clinical outcomes (e.g. different evolution of the disease in skin and in skeletal muscle in dermatomyositis). This argues for the establishment of IFN-signatures that are tissue-specific/cell-specific (Fig. 2). Therefore, the IFN-signature requires an international consensus for its standardization in order to generalize its practice.

Standardized tissue-specific IFN-signature will allow to refine IIM subgroups, as well as to define responding patients who may be eligible for specific therapies (e.g. JAK/Stat inhibitors [38[■]]). On the other hand, transcriptomic analysis may also link different disease pathogenesis. For example, the IFN-signature in dermatomyositis, SLE and HSV-2 (herpes simplex virus-2) skin samples is remarkably similar, suggesting that skin alteration of these disorders has a common pathophysiology [12].

The IFN-signature, through the analysis of ISG transcripts, appears to be a precious tool for the understanding of IIM pathogenesis, as well as for refining the clinical status, monitoring the evolution and evaluate therapeutic intervention of IIM patients.

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Conflicts of interest

There are no conflicts of interest.

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1.3.2.5 Clinical proof of concept and correlation of disease activity and IFN signature

Multiple studies have stated that the IFN signature correlates with the disease activity (Baechler et al., 2007; Greenberg et al., 2012; Huard et al., 2017; Reed et al., 2012). To illustrate this point, Baechler et al have set up an IFN score, based on the expression of 43 ISGs in PBMC, which appears to be correlated with a simple disease activity score based on muscle strength testing, muscle enzyme elevation, skin involvement and patient's report of functional assessment (Baechler et al., 2007). Similarly, transcriptomic analysis of skin shows that, patients with inactive skin DM cluster with the healthy controls, in contrast to active skin DM patients (Wong et al., 2012). Similarly, Gitiaux et al found a correlation between disease vascular severity score and IFN- α levels in lysates of MPCs (but no correlation with IFN signature) (Gitiaux et al., 2016). In another work, Ishikawa et al reported that serum levels of IFN- γ were significantly higher in DM with rapidly progressive ILD (DM-RP ILD), while serum level of IFN- α was increased in all DM subgroup (Ishikawa et al., 2018).

After demonstration of IFN involvement in DM pathogenesis, the logical next step was to assess its therapeutic consequences. The first successful use of JAK/STAT inhibitor in DM patients reported appeared due to serendipity. Indeed, a patient suffering from refractory DM with partial efficacy of 3 lines of immunosuppressive therapy developed fever, marked splenomegaly and thrombocytopenia (Hornung et al., 2014). Testing for the Janus kinase 2 mutation JAK2 V617F was positive, which resulted in a diagnosis of post-polycythemia vera myelofibrosis, and the initiation of a treatment with JAK inhibitor (ruxolitinib). Ruxolitinib is a selective inhibitor of JAK1 and JAK2 and was developed for the treatment of myelofibrosis. The pharmacological target of the molecule is to block the IFN- β -induced STAT1 (Hornung and Wenzel, 2014). Unexpectedly for the patient, DM rapidly improved during ruxolitinib treatment. The patient regained muscle strength and body weight, and her skin lesions completely resolved (Hornung et al., 2014). Usefulness of JAK inhibitor in refractory DM has been corroborated by several other case reports (Dj et al., 2016; Hornig et al., 2018; Ladislau et al., 2018; Wendel et al., 2019). In addition, a translational study by Ladislau et al reported that type I interferon could abolish myogenic differentiation of myoblasts *in vitro*, which could be counteracted with Ruxolitinib. In the same study, authors reported 4 patients with refractory

DM whose diseases were improved by ruxolitinib, including skin lesions, muscle weakness as well as a reduced serum type I interferon levels and ISG score (Ladislau et al., 2018).

Considering the recurrent observations of IFN involvement in IIM pathogenesis, the question of targeting IFN appears justified. Examples in other diseases have to be considered. Notably, in the case of psoriasis patients who present a prominent IFN signature (Yao et al., 2009), inhibition of the IFN-I pathway seems to have no clinical effect (Bissonnette et al., 2010). Also, the link between *in vitro* and *in vivo* data remains complex and requires to be cautious in the extrapolation from bench to patients. This highlights the need for a better understanding of the components and mechanisms of the IFN response in IIM. Notably, the interplay of IFN I and II in IFN signatures observed in blood and tissues of patients with IIM has to be clarified (Hall et al., 2012).

1.3.3 Mechanism of IFN in DM pathogenesis

IFN has been involved in DM pathogenesis at various levels, such as circulating IFN-I, IFN-I inducible genes, IFN-I induced proteins and IFN-I related cytokines (Arshanapalli et al., 2015). Several mechanisms have been hypothesized for IFN-I involvement in DM pathogenesis.

First, cells secreting IFN are present in IIM muscle. Indeed, mature plasmacytoid DCs (pDCs) identified CD4, CD123 and CD83 are found in muscle and skin of jDM patients (López de Padilla et al., 2007). Interestingly, pDCs are known to be involved in innate immune responses and to produce IFN-1 α and β (Colonna et al., 2004), making these cells at a pivotal role in the initiation of self-immune response but also for the maintenance of autoimmune lesions. Li et al formulated the hypothesis that endogenous production of type I IFN in DM may be generated by pDCs, mainly through the Toll like receptor (TLR)-9 pathway, and in part by TLR-7 (Li et al., 2015).

Other sources of IFN-I have been identified, such as the myofiber itself. Suarez-Calvet et al demonstrated that in microdissected pathological muscle fibers from DM, retinoic acid-inducible gene 1 (RIG-I, DDX58) and the novel antiviral factor DDX60, which promotes RIG-I-mediated signaling, were significantly up-regulated, as well as IFIH1 (MDA5) and TLR3 (Suárez-Calvet et al., 2014). Immunohistochemistry confirmed RIG-I over-expression in pathological muscle fibers, specifically in DM as compared with other IIM and controls. *In vitro* investigations demonstrated that stimulation of human myotubes with a RIG-I ligand (5' ppp-dsRNA) induced a significant secretion of IFN- β and an up-regulation of MHC-I, RIG-I

and TLR3 expression by IFN- β -dependent and TLR3-independent mechanisms. These results indicate that RIG-I pathway activation induces MHC-I over-expression, secretion of IFN- β and a positive feedback on RIG-I in myofibers (Suárez-Calvet et al., 2014). In another work, immature muscle precursors were identified as a local source of IFN- β , and could be implicated in MHC-I over-expression through TLR3 pathway (Tournadre et al., 2012).

Then, IFN as a factor for IIM initiation and maintenance through IFN-I induced proteins. MDA5 and RIG-I, two IFN-Is induced proteins may similarly play a role in the positive feedback loop involved in DM. Indeed, MDA5 also functions as a cytosolic receptor for viral double-stranded RNA that once activated, stimulates an innate immune response including production of IFN-I (Chistiakov, 2010; Kato et al., 2008; Nakashima et al., 2010; Sato et al., 2009). Both MDA5 and RIG-I have been demonstrated to cause IFN-I production in virally infected cells (Ikegame et al., 2010), and the association of a positive feedback loop of IFN-I production and viral infection raises the controversial question of a viral hit that may initiate or perpetuate the autoimmune phenomena.

Lastly, another putative pathway involves IFN-I related cytokines, such as monocyte chemo attractant protein-1 (MCP-1/CCL2) which has been found at elevated levels in DM. Staining for MCP-1 showed a predilection for endothelial cells near areas of active inflammation, not only in DM (De Bleecker et al., 2002) but also in all IIM (De Paepe et al., 2009). This endothelial localization suggests that MCP-1 may play a role in complement deposition in the microvasculature of affected muscles (Jain et al., 2011). Indeed, DM muscle biopsies express C5-b9 on endothelial cells which may play a role in capillary damage (Kissel et al., 1986, 1991). The depletion and damage of capillaries may result in hypoxia and in the subsequent damages to myofibers seen in DM (Dalakas and Hohlfeld, 2003). Abnormal capillary morphology and capillary loss is an early manifestation of DM, often seen prior to inflammatory infiltrates (Emslie-Smith and Engel, 1990; Kissel et al., 1986).

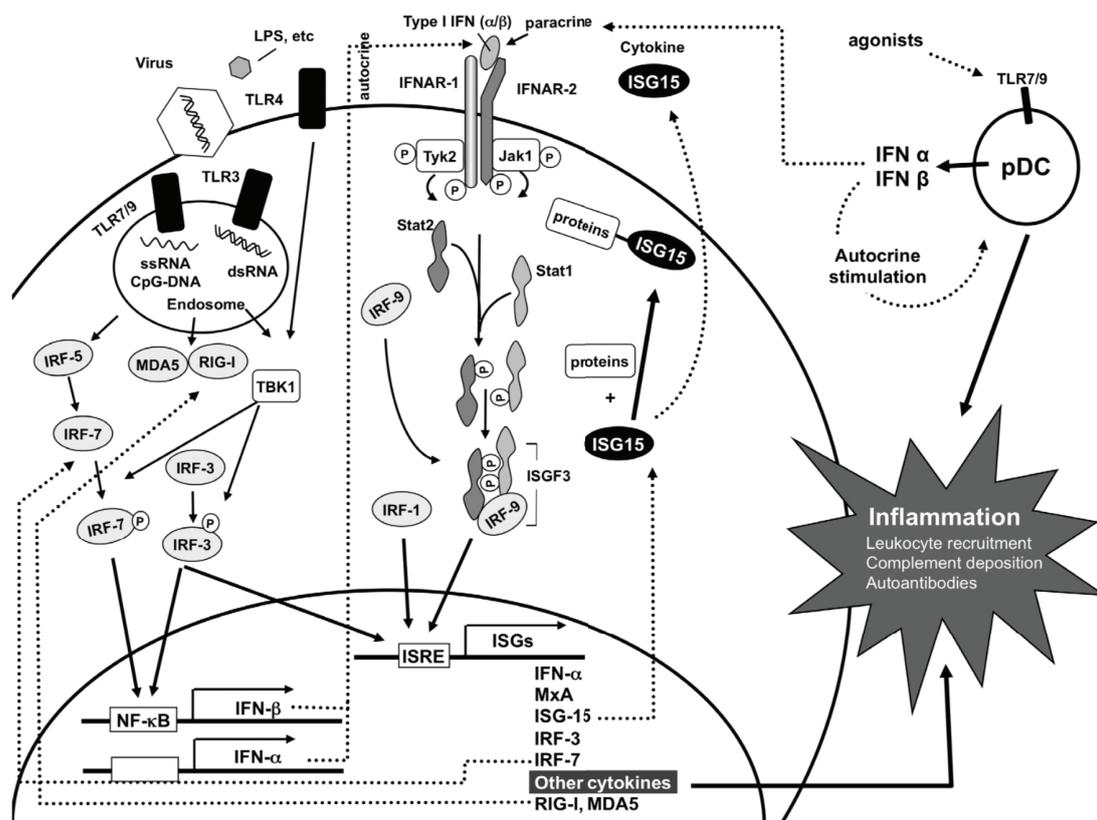


Figure 2. Interferon involvement in DM pathogenesis. Type I interferon pathway. Mechanistic pathway showing production of IFN-Is, signaling pathway induced by IFN α and - β , and downstream effects including stimulation of genes, production of proteins, and resulting inflammation. IFNAR, IFN α receptor; JAK, Janus Kinase; Tyk2, Tyrosine Kinase 2; STAT, Signal Transducer and Activator of Transcription; ISGF3, Interferon Stimulated Gene Factor 3; IRF, Interferon Regulatory Factor; ISRE, Interferon Stimulated Response Element; ISGs, Interferon Stimulated Genes; ss, single-stranded; ds, double-stranded; LPS, Lipopolysaccharides; TLR, Toll-like receptor; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; TBK1, TANK-binding kinase 1. Activation and positive feedback pathways of the type I IFN receptor are shown with dotted arrows. From Arshanapalli et al 2014

1.3.4 IFN and autoimmunity

The mechanisms of type I IFN-induced autoimmunity has been broadly discussed, as IFNs are described at elevated levels in autoimmune disorders, such as systemic lupus erythematosus.

This has been attributed in part to increased DC activation and antigen uptake/presentation from apoptotic cells, as well as increased production of autoantibodies (Jego et al., 2003; Rizza et al., 2010).

In a study conducted by Eloranta et al, immune complexes containing anti-Jo-1 or anti-Ro 52/anti-Ro 60 autoantibodies and RNA were identified as endogenous IFN- α inducers that activate IFN- α production in pDCs (Eloranta et al., 2007). Another study showed that nucleic acid associated antibodies (including some antibodies already associated with IIM even if there are not specific: anti Ro, La, Sm, and RNP) may stimulate the production of active IFN- α in jDM (Balboni et al., 2013). This study demonstrated a correlation between the presence of antibodies against RNA-containing autoantigens and both serum IFN α activity and the capacity to generate interferogenic immune complexes. This led to hypothesize that RNA-containing autoantigens, including Ro, La, Sm, and RNP, play a critical role in the activation of the type I IFN system in jDM.

Piper et al demonstrated that jDM patients with active disease have a significantly expanded immature transitional B cell population, which correlated with the IFN-I signature. Activation through TLR7 and IFN α may drive the expansion of immature transitional B cells in jDM and may skew the cells toward a pro-inflammatory phenotype (Piper et al., 2018).

Despite the above-mentioned evidence of immune system implication, many pieces miss to complete the puzzle. This could be one of the reasons for partial efficacy of immunosuppressive treatments. Actually, this unfortunate consequence could raise new interrogations, such as the potential unbalanced process between muscle damage and repair. Indeed, skeletal muscle of IM patients is characterized by inflammatory infiltrates and signs of myofiber regeneration that is operated by MPCs to replace damaged myofibers.

2. Adult myogenesis / muscle regeneration (cellular and inter-cellular features)

Mature muscle cells, also called myofibers, are not able to divide. However, skeletal muscle is a tissue which repairs ad integrum after a damage thanks to the involvement of muscle stem cells.

2.1. Muscle stem cells

Muscle stem cells (MuSC), also called satellite cells, are quiescent mononuclear cells, and were discovered through the advent of electronic microscopy by Alexander Mauro in the 1960's (Mauro, 1961). Their name "satellite cells" comes from their particular location beside the myofiber, between the plasma membrane of the myofiber and the basal lamina surrounding the same myofiber (Figure 3A).

The functions of MuSC have been identified in 1975, through the *in vitro* observation of a single myofiber regeneration (Bischoff, 1975). By contrast microscopy, it was possible to see *in vitro* cell proliferation at the surface of the isolated myofiber. In 1977, analysis by electron microscopy of the satellite cells during muscle regeneration showed for the first time that MuSC are activated (and rename myogenic precursor cells (MPC) then differentiate into myoblasts, and lastly fuse in myotubes in order to repair the myofiber (Sakai, 1977). Isolation of satellite cells and histological analyses allowed the discovery of the marker Pax7, which is highly specific of satellite cells in skeletal muscle (Seale et al., 2000) (Figures 3B, 3C). Pax7 belongs to the family of homeodomain paired box transcription factors that are involved in development.

Pax7 is crucial for the establishment of satellite cell population in muscle (Yin et al., 2013). However, other markers are expressed in mouse satellite cells such as CD34, CXCR4, syndecan4, Vcam1 and β 1-integrin, α 7-integrin (Figure 3D) (Yin et al., 2013). More recently, the role of Pax7-expressing cells in muscle repair was evaluated thanks to an inducible cre-lox system in the mouse, demonstrating that the Pax7-expressing cells, i.e. satellite cells are absolutely required for muscle regeneration (Lepper et al., 2011; Murphy et al., 2011; Sambasivan et al., 2011).

Interestingly, few weeks after an injury, satellite cells are present in the muscle, back to their location under the basal lamina, indicating that cells do participate to skeletal muscle regeneration, as well as they return to quiescence in order to reform the pool of stem cells

(Collins and Partridge, 2005; Montarras et al., 2005; Sacco et al., 2008). This phenomenon of self-renewal is essential for the long-term maintenance of muscle homeostasis.

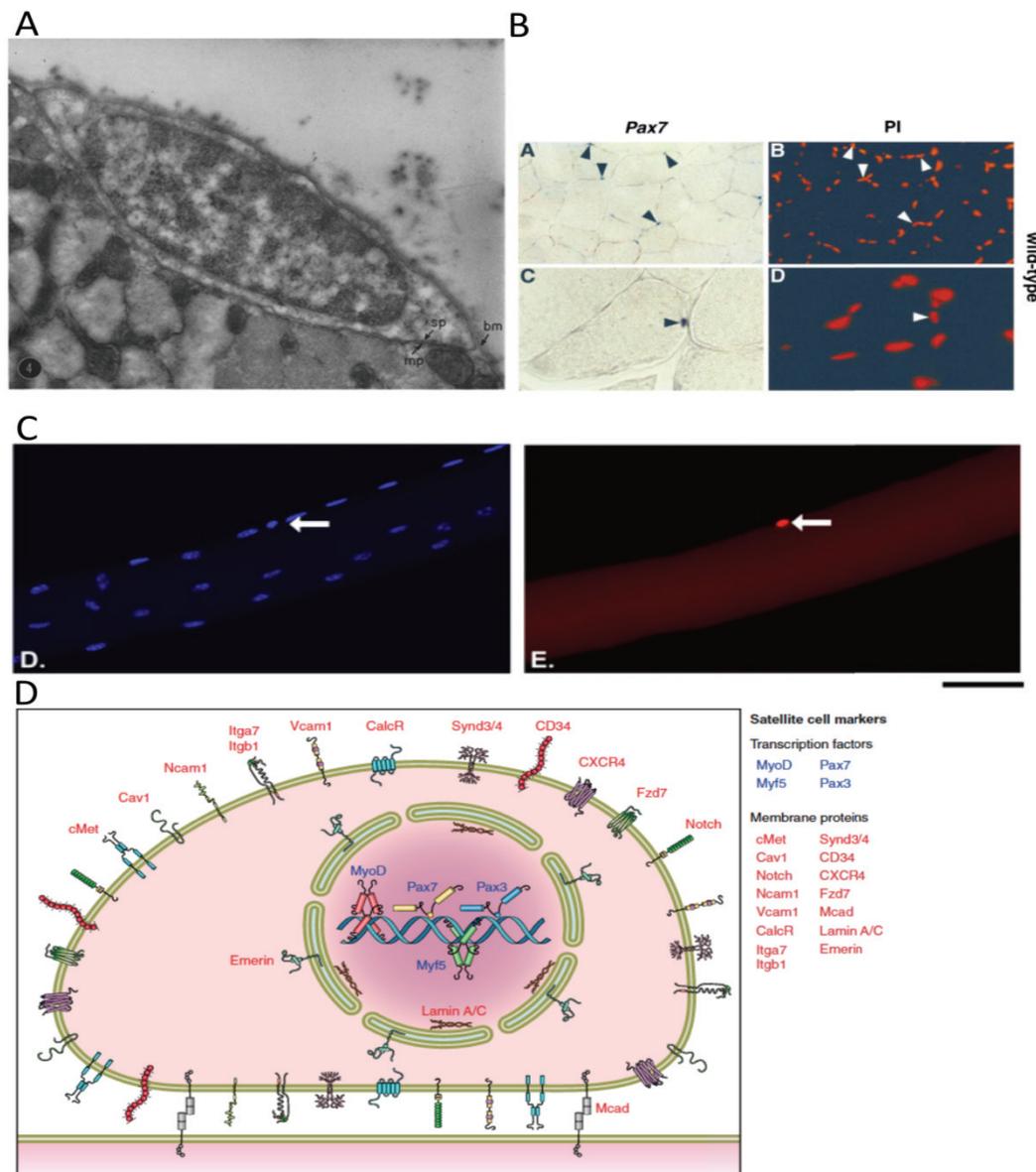


Figure 3. Satellite cells express the homeodomain paired box 7 (Pax7) (A) First picture of a satellite cell in electron microscopy of a rat muscle (Mauro, 1961). (B) In situ hybridization of Pax7 mRNA (blue staining, left picture) reveals that few isolated nuclei present around the myofibers express this transcription factor as compared with the total number of nuclei in the tissue section (red staining, right picture) (Seale et al., 2000). (C) Immunofluorescence of Pax7 indicates that Pax7pos (highly red cell, right picture) are located around the myofiber. DAPI staining (left picture), which stains all the nuclei of the myofiber, shows the small proportion

of nuclei expressing Pax7 (Pasut et al., 2013). (D) Satellite cells express several markers, which are used for their identification and isolation (Yin et al., 2013).

MuSCs are present around myofibers in a quiescent state and are located in the so-called satellite cell niche. Although not well-characterized yet, this structure is essential for the maintenance of satellite cell quiescence. Indeed, protein interactions between the muscle fiber and satellite cells were identified to maintain satellite cells in a quiescent state such as those implicating M-cadherin and Notch ((Baghdadi et al., 2018; BJORNSON et al., 2012; Evano and Tajbakhsh, 2018; Kuang and Rudnicki, 2008; Mourikis et al., 2012). Interactions between satellite cells and extra-cellular matrix (ECM), which composes the basal lamina, also participate to maintain satellite cell quiescence thanks to integrins (Evano and Tajbakhsh, 2018; LaBarge and Blau, 2002). Other cell types participate to the homeostasis of the satellite cell niche. Endothelial cells are found to be very close to satellite cells in resting condition (Abou-Khalil et al., 2009). After an injury, satellite cell niche alteration is critical for satellite cell activation (Yin et al, 2013) (Yin et al., 2013).

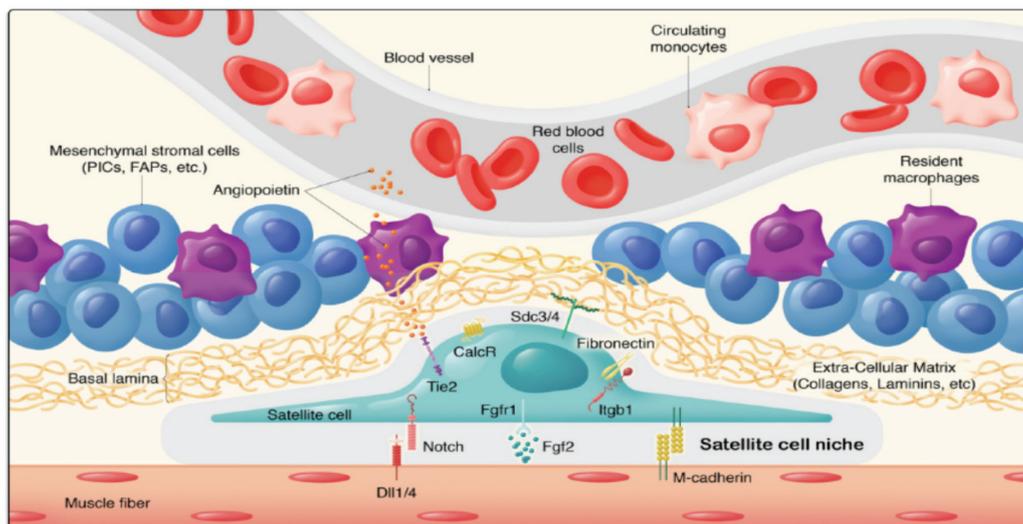


Figure 4. The satellite cell niche. In resting muscle, quiescent satellite cells are located in a niche. Myofiber itself is involved in the maintenance of the quiescence by Notch and cadherin signaling. Other factors are implicated in the maintenance of the quiescence such as angiopoietin or Fgf2. Macrophages, mesenchymal stromal cells and endothelial cells are close neighbors of the niche (From Evano and Tajbakhsh, 2018).

2.2. Myogenesis and myogenic regulators

After skeletal muscle lesion, satellite cells leave their quiescent state and start to proliferate (Yin et al., 2013). These activated satellite cells, now called MPCs (myogenic precursor cells) undergo the myogenesis process in order to achieve muscle repair. This process requires a careful orchestration of activation and proliferation of MPCs and their subsequent differentiation and fusion into multinucleated myotubes, which leads to the reconstitution of a functional contractile apparatus. The complex behavior of MPCs during skeletal muscle regeneration is tightly regulated through the dynamic interplay between intrinsic factors within MPCs and extrinsic factors constituting the muscle stem cell niche/microenvironment. This process follows different steps: activation, proliferation, commitment in differentiation, fusion into myotube and lastly, maturation into myofiber. Myogenesis is mainly driven by the family of the Myogenic Regulatory Factors (MRFs) (Musarò, 2014), composed of 3 main factors: Myf5, MyoD and myogenin. MRFs are differentially expressed at the various steps of myogenesis. (Figure 5)

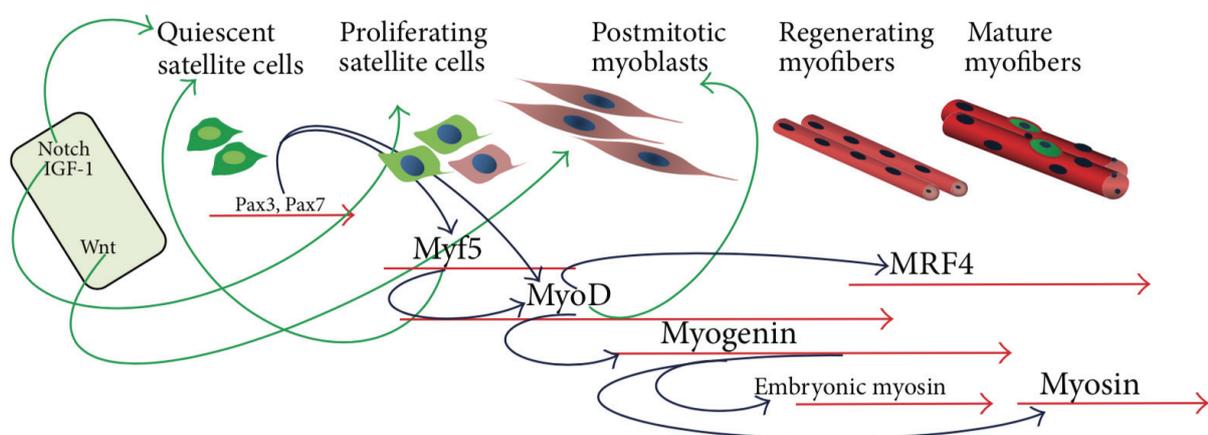


Figure 5. MRF expression during myogenesis. Illustration of the lineage progression of adult myogenesis during muscle regeneration. Environmental cues (Notch, IGF-1, Wnt, etc.) influence the activity of satellite cells (green arrows). Notch and IGF-1 signaling stimulate the proliferation of satellite cells, whereas Wnt signaling is involved in the transition from proliferation to the differentiation phase of myoblasts. Notch signaling is also necessary for the maintenance of the quiescent state of satellite cells. Depending on MyoD activity, satellite cells can follow one of two fates: they may maintain Myf5 expression while downregulating MyoD

and self-renewing; alternatively, they maintain MyoD expression and differentiate. Quiescent satellite cells express Pax3 and Pax7. Pax7 regulates MyoD and Myf5 expression (blue arrows). Myf5 regulates the expression of MyoD, which in turn promotes the expression of myogenin and MRF4 (blue arrows). Myogenin promotes the expression of Myosin (blue arrows) (From Musaro et al., 2014).

2.2.1 Activation of MuSCs

In resting muscle, MuSCs are quiescent. The MuSC cell cycle is arrested in the G0 phase. This quiescent state is maintained by the niche structure and environment. When the niche is altered by muscle lesion or exercise, MuSCs exit from quiescence and activate to give rise to myoblasts or myogenic precursor cells (MPCs) (Yin et al., 2013). An intermediate state between the quiescent state and the activated state was recently described and called the G-alert, in non-injured muscles of a mouse that was injured in a remote muscle. MuSCs in G-alert may be considered as pre-activated cells as they are able to activate faster than in G0 state after an injury (Rodgers et al., 2014). Regarding the molecular pattern, activated MuSCs express Pax7. Quickly after activation, they also express the MRF Myf5 (Figure 5). Myf5 mRNA was shown to be expressed by quiescent stem cells but the mRNA is sequestered in a structure called messenger ribonucleoprotein granules. During activation state, these granules are dissociated and allow the rapid translation of Myf5 mRNA (Crist et al., 2012).

MuSC activation depends on multiple pathways, among them some have been further studied. Two extrinsic secreted factors have been studied and appear linked: hepatocyte growth factor (HGF) and nitric oxide (NO). HGF is a heterodimer of α and β subunits, both of which are processed from a single-chain inactive precursor (pro-HGF). HGF is critical for cell growth, migration, and organ morphogenesis through its mitogen, motogen, and morphogen activities (Ohnishi and Daikuhara, 2003). In skeletal muscle, HGF functions in the early phase of muscle regeneration and is one of the key activators of satellite cells (Miller et al., 2000). In intact muscle, low levels of HGF mRNA can be detected in satellite cells but not in fibroblasts, suggesting an autocrine function of HGF (Sheehan et al., 2000). However, the majority of active and inactive forms of HGF are sequestered by heparan sulfate proteoglycans (HSPGs) within the basal lamina (Sheehan et al., 2000). Upon injury, the transcription of HGF is increased in

proportion to the degree of injury, and an active form of HGF is released from the extracellular matrix.

NO is a diffusible small molecule, which can freely pass through cell membranes. It can be produced by nitric oxide synthase (NOS) in diverse cell types, including epithelial cells, endothelial cells, fibroblasts, hepatocytes, macrophages, and skeletal muscle cells (Bogdan, 2001). Currently, three NOS isoforms have been identified: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (Pacher et al., 2007). *In vitro* and *in vivo* data have revealed that release of nitric oxide synthase (NOS) from the basal lamina after myofiber damage leads to the production of NO, which in turn activates matrix metalloproteinases and release HGF from local HSPGs (Tatsumi et al., 2006). During regeneration, NO induces satellite cell activation, as evidenced by the inhibitory effect of L-arginine methyl ester, a NOS inhibitor (Anderson, 2000). NOS inhibition prevents the binding of HGF to its c-Met receptor on satellite cells, whereas treatment with L-arginine, the NOS substrate, resulted in an increase in satellite cell activation. Accordingly, iNOS^{-/-} mice display delayed satellite cell activation following muscle injury, although injured muscles were still able to regenerate (Anderson, 2000). The function of NO-dependent satellite cell activation stems from the ability of NO to promote the release of HGF from the ECM (Yamada et al., 2006, 2008).

2.2.2 Proliferation of MPCs

After activation, MPCs enter in a phase of proliferation and will reach the cellular density required for the next steps of the repair process. On a molecular side, Myf5 mRNA that was present under a sequestered form during quiescence re-localizes to induces proliferation. Indeed, in the *myf5*^{-/-} mouse model, MPC proliferation rate is decreased (Ustanina et al., 2007). Myf5 is a transcription factor that controls the expression of genes required for proper myogenesis (Conerly et al., 2016) (Conerly et al., 2016). The MRF MyoD is also expressed by proliferating cells and this factor is important for the differentiation step. Moreover, other signaling pathways are required for MPC proliferation such as Notch and Wnt signaling pathway (Conboy and Rando, 2002; von Maltzahn et al., 2012).

Using a model of isolated myofibers maintained in culture, Zammit et al have demonstrated that satellite cell progeny adopted divergent fates: although most differentiate, others regain a

phenotype characteristic of quiescent satellite cells. This variation in satellite cells fates appeared tightly regulated by MRF expression, noticeably Pax7 and MyoD (Zammit 2004) (Zammit et al., 2004).

2.2.3 Differentiation of MPCs into myocytes

The end of proliferation is marked by the differentiation of MPCs. MPCs enter into differentiation as a consequence of multiple modifications of intrinsic and extrinsic regulations (Joulia-Ekaza et al., 2006). Moreover, the onset of differentiation is associated with a transition from Notch signaling to Wnt signaling in MPCs, with an increased expression of Wnt in the tissue and an increased responsiveness of MPCs to Wnt (Brack et al., 2008). Crosstalk between these two pathways occurs via GSK3b, which is maintained in an active form by Notch but is inhibited by Wnt in the canonical Wnt signaling cascade. Thus, the temporal balance between Notch and Wnt signaling orchestrates the precise progression of MPCs along the myogenic lineage pathway during adult myogenesis (Brack et al., 2008).

Regarding MRF, when the dividing process stops, differentiating cells shutdown the expression of Pax7 (Figure 6) (Tedesco et al., 2010). Actually, a ratio between Pax7 and MyoD was implicated for the induction of the differentiation (Olguin et al., 2007). A low Pax7/MyoD ratio was suggested to induce the commitment of proliferating cells to differentiation (Olguin et al., 2007). Studies have shown that MPCs deficient for MyoD do not differentiate properly showing the essential role of this transcription factor in myogenic cell differentiation (Wood et al., 2013; Yablonka-Reuveni et al., 1999). While MyoD appears to be necessary for the early phase of differentiation commitment, the MRF myogenin is expressed at the end of the differentiation process to specifically trigger the expression of myogenic genes (Bentzinger et al., 2012). Myogenin expression induces cell cycle stop through p21 activation (Chargé and Rudnicki, 2004). Myogenin was originally discovered in 1989 to allow the expression of myogenic markers in mesenchymal cells after transfection (Wright et al., 1989). Moreover, myogenin null-mice show a defect in embryonic myogenesis showing the importance of this factor for myogenesis (Hasty et al., 1993). The sequential and timely expression of the myogenic regulators is primordial for MuSC differentiation and skeletal muscle regeneration.

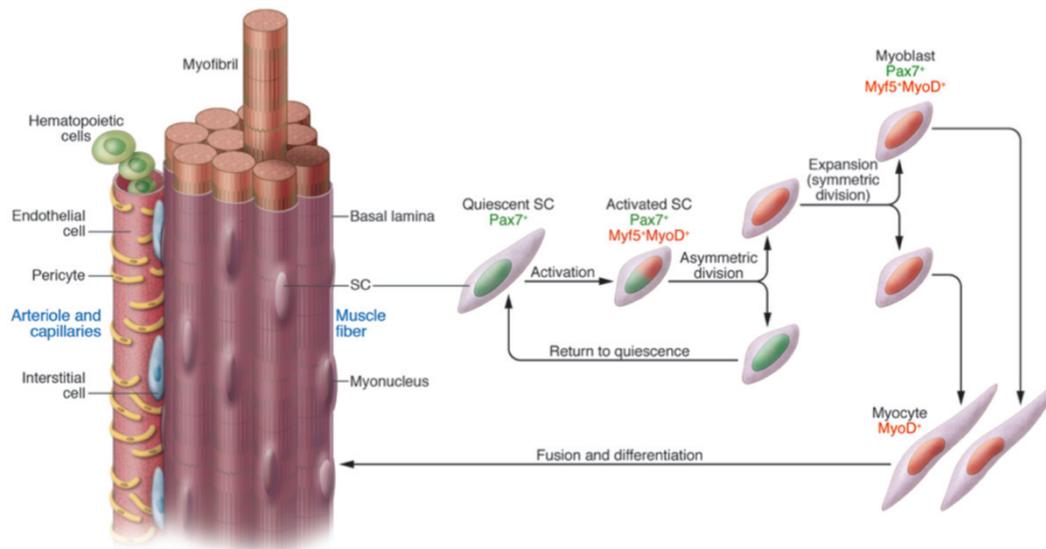


Figure 6. Differentiation of muscle stem cells. After an injury, Pax7⁺ quiescent cells (green cells) are activated. These activated cells express Myf5 and MyoD (green and red cells). After proliferation, cells differentiate into myocytes and stop to express Pax7 (red cells). Then, they fuse to repair myofibers. A proportion of myogenic proliferating cells (green and red cells) returns to quiescence in order to replenish the pool of muscle stem cells (back to green cells) (Tedesco et al., 2010).

2.2.4 Fusion of myocytes into myotubes

Differentiated myoblasts, which are called myocytes fuse with each other to form new multinucleated myofibers. The fusion process consists of 5 different steps: myocyte alignment, cell to cell recognition, inter-cell adhesion, plasma membrane apposition and finally fusion itself, i.e. merging of the plasma membranes (Figure 7). The recognition of the fusion partner is highly specific since myogenic cells fuse only with myogenic cells. The newly formed myofiber is initially centri-multinucleated and secondarily nuclei migrate toward the periphery of the myotube. Moreover, fusion events involve cytoskeletal reorganization and implicate the regulation of the plasma membrane tension between the two-fusing partner-cells depending on actin and myosin (Kim et al., 2015).

Membrane protein content is important to allow fusion. Integrins and cadherins are essential in this process (Chargé and Rudnicki, 2004; Schwander et al., 2003). Recently, a master protein involved in fusion was identified, myomaker. Myogenic cells depleted for myomaker are not

able to fuse and in myomaker-null mice, embryos die perinatally due to the absence of muscle fusion (Millay et al., 2013).

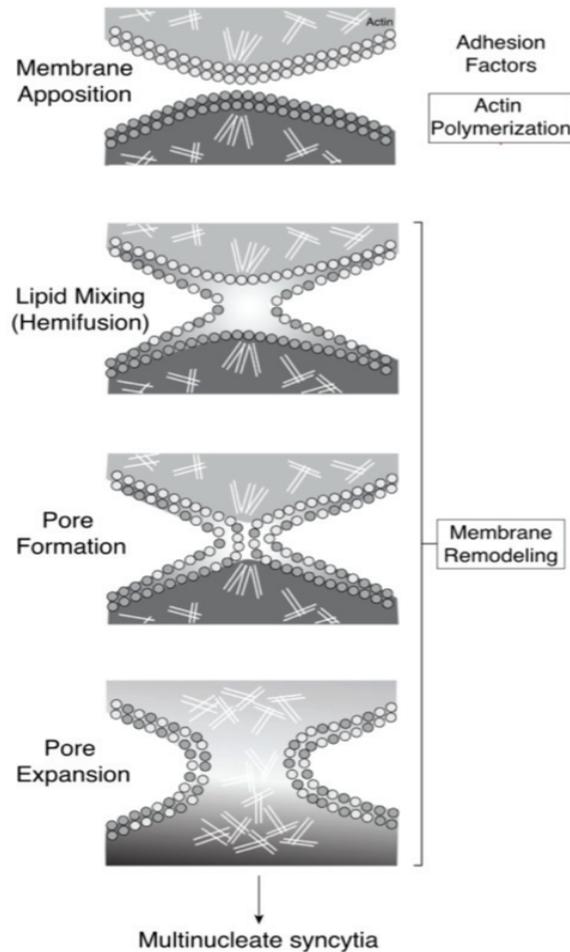


Figure 7. Mechanism of myoblast fusion. The fusion of differentiated myoblasts is essential to form new myofiber syncytia or to fuse with existing myofibers. Fusion requires specific apposition of the cells. Reorganization of the cytoskeleton is essential. Moreover, specific proteins are crucial for myoblast fusion, such as myomaker, a transmembrane protein (Sampath et al., 2018).

For human, mice and other species, isolated MuSC cultured in vitro will recapitulate the in vivo myogenic program, requiring no extrinsic stimulation. Activation of the MuSC has been induced by cells isolation. This support that myogenic program has to be considered as a very potent process.

2.2.5 Myogenesis and cellular environment

As described below, the myogenic program drive intrinsic cellular orchestration through tightly regulated molecular pathway. Despite these considerations, extrinsic factor may alter this repair process. Indeed, during muscle tissue repair, MPCs are involved in a synergistic process implying MPCs, endothelial cells, fibro-adipogenic progenitors (FAPs) and immune cells among which are macrophages.

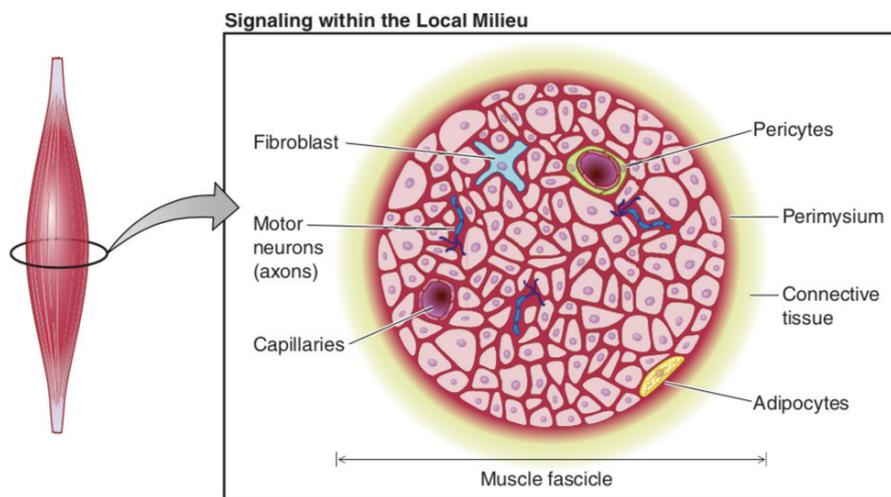


Figure 8. Various cell types in the muscle tissue. The muscle fascicle defines the extremities of the local milieu an environment that is more diverse compared with the immediate niche due to the heterogeneity of cell types and signaling factors. The local milieu surrounding the satellite cell is made up of other myofibers in addition to interstitial cells, capillaries, and neuromuscular junctions. Each of these cell types (endothelial cells, fibroblasts and immune cells such as monocytes/macrophages or neutrophils) influences the surrounding environment and thereby affects the state of the satellite cell. (From Yin et al., 2013).

2.2.5.1. Macrophages

Muscle injury triggers the release of chemoattractant molecules in the blood stream to recruit circulating leukocytes. The main route of monocyte entry into the injured muscle is the chemokine CCL2 (MCP1)/CCR2 axis. Once in the tissue, monocytes differentiate into pro-inflammatory macrophages. Their functions are very specific. First, macrophages allow the

clearance of cell debris by phagocytosis. They also induce survival and growth of MPCs by establishing direct cell-cell contacts, and inhibit their differentiation and fusion (Chazaud et al., 2003; Sonnet et al., 2006). Pro-inflammatory macrophages secrete factors such as IL-6, IL-1 β or Vascular endothelial growth factor (VEGF) that stimulate MPC proliferation (Arnold et al., 2007; Saclier et al., 2013). Finally, pro-inflammatory macrophages induce Fibro/adipogenic progenitors (FAP) and fibroblast apoptosis to avoid excess of matrix deposition (Juban and Chazaud, 2017; Lemos et al., 2015).

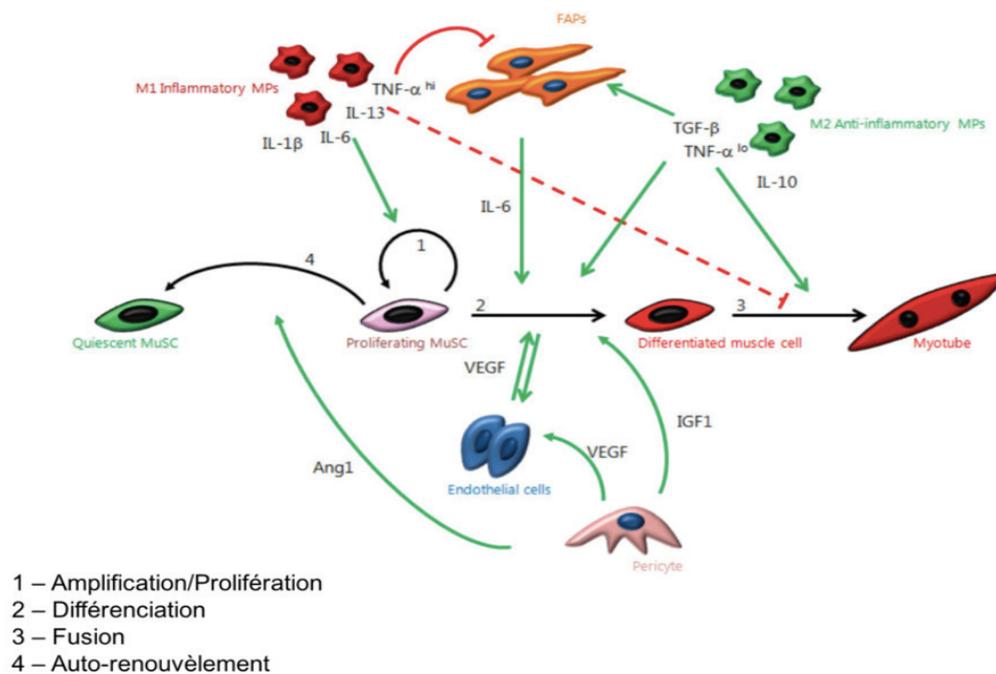


Figure 9. Cellular interaction during myogenesis process. During post-injury myogenesis, satellite cells proliferate then differentiate, and a small percentage of the cells will return to quiescence (self-renewal process). Extrinsic factor, notably various type of cells (macrophages, endothelial cells, pericytes, and Fibro-Adipogenic Progenitors (FAPs), and several cytokines, will interact during each step of myogenesis. (Adapted from Théret 2016)

At the time of resolution of inflammation, pro-inflammatory macrophages shift their inflammatory status toward an anti-inflammatory phenotype. Signaling pathways involved in this switch start to be documented. For now, 3 main intracellular pathways have been described: AMPK, p38/MKP1, and CREB-C/EBP β . However, it was shown that phagocytosis is essential for the acquisition of an anti-inflammatory phenotype. Indeed, the expression of pro-inflammatory markers is reduced and the expression of anti-inflammatory markers is increased in macrophages that have ingested apoptotic cells (Fadok et al., 1998; Freire-de-Lima et al.,

2006; Weigert et al., 2006). Anti-inflammatory macrophages exert various roles on several cell types in regenerating skeletal muscle. They induce both differentiation and fusion of MPCs as well as growth of the newly regenerated myofibers (Arnold et al., 2007; Mounier et al., 2013; Saclier et al., 2013). Anti-inflammatory macrophages promote ECM remodeling by inducing fibroblast survival and collagen production through the secretion of TGF- β (Juban and Chazaud, 2017). They also induce angiogenesis: *in vitro* experiments showed that anti-inflammatory macrophages induce endothelial cell sprouting and differentiation, triggering vessel formation concomitantly to myogenesis, through the delivery of specific effectors, such as Oncostatin M (Latroche et al., 2017). Accordingly, CCR2 KO mice exhibit defect of vascularization in regenerating muscle (Ochoa et al., 2007). Thus, anti-inflammatory macrophages act on various cell types by simultaneously promoting growth of newly formed muscle cells, remodeling of ECM and revascularization in order to obtain a complete functional recovery of the muscle.

2.2.5.2 Endothelial cells

As a consequence of its high metabolic needs, muscle is an opulently vascularized tissue, including vessels and capillaries. It was evidenced that MPCs and endothelial cells are localized in close proximity (Christov et al., 2007). Interestingly, in cocultures, endothelial cells promote myoblast proliferation by secreting a panel of growth factors, such as IGF-I, HGF, FGF, platelet-derived growth factor-BB (PDGF-BB), and VEGF (Christov et al., 2007). It is also well known that angiogenesis and myogenesis proceed at the same time during muscle regeneration (Luque et al., 1995), and that both processes are regulated by some common factors such as VEGF (Chazaud et al., 2003; Christov et al., 2007; Latroche et al., 2017). In addition, it was found that periendothelial cells (smooth muscle cells) induce a subset of myoblasts (presumably ready to self-renew) to return to the quiescent state (Abou-Khalil et al., 2009).

2.2.5.3 Fibro-Adipogenic Progenitors

FAPs are resident in muscle tissue but arise from a distinct developmental lineage than MuSCs. These cells remain quiescent in intact muscle. In response to muscle injury FAPs proliferate to transiently establish an environment that enhances myogenic differentiation (Joe et al., 2010).

Interestingly, FAP behavior is also regulated by infiltrating inflammatory macrophages. Through their expression of tumor necrosis factor (TNF) α , pro-inflammatory macrophages induce apoptosis of FAPs. However, in case of chronic damages, such as those occurring in mdx mice, macrophages express high levels of transforming growth factor β 1 (TGF- β 1), which prevents FAP apoptosis and induces their differentiation into matrix-producing cells.

3. IIM and muscular regeneration: Does muscle injury evolve chronically as a result of muscle regeneration impairment?

Despite all the immunosuppressive drugs prescribed, IIM patients rarely fully recover their initial strength (Bronner et al., 2006; Clarke et al., 1995). The explanation of this residual muscle dysfunction remains unclear. Some authors have hypothesized that chronic muscle dysfunction could be due to possible defects in muscle repair mechanisms, disuse of muscle, or replacement of muscle tissue by fibrotic tissue or fat, with an influence of both disease-related factors and glucocorticoid treatment (Cseri et al., 2015; Loell and Lundberg, 2011).

Muscle regeneration process is well understood in physiological condition. Interestingly, multiple immune and non-immune dysfunctions in IIM may have an impact on the regeneration process itself (Loell and Lundberg, 2011). Several proinflammatory molecules found in muscle tissue of IIM patients may impair muscle repair and recovery. In addition, some of the usual treatment of IIM are known to impact myogenesis, foremost corticosteroids. The delicate balance between the downregulation of proinflammatory molecules by immunosuppressive treatments and their inhibitory effects on muscle fiber regeneration requires further consideration.

3.1. Impairment of muscle regeneration in IIM

3.1.1 Muscle regeneration process in IIM

In the early identification of IIM disease group, skeletal muscle regeneration has been studied, and appears frequently associated with necrosis and myophagy. Occurrence of regenerative basophilic fibers containing internal nuclei as a mark of ongoing myogenesis process is widely described (Winter and Bornemann, 1999). Additional characterization of regenerative myofibers are usually reported by the expression of CD56 or neonatal myosin (Gitiaux et al., 2016; Musarò, 2014).

Neural cell adhesion molecule (NCAM), also called CD56, is a homophilic binding glycoprotein expressed on the surface of neurons, glia and skeletal muscle. In tissue sections, CD56 is present on the sarcolemma of the satellite cells and the regenerating or newly denervated muscle fibers. Once a fiber becomes innervated NCAM becomes localized to the neuromuscular junction. In tissue cultures, CD56 labels myoblasts (Winter and Bornemann, 1999). Developing skeletal muscles express unique myosin isoforms, including embryonic and neonatal myosin heavy chains, coded by the myosin heavy chain 3 (MYH3) and MYH8 genes,

respectively, and myosin light chain 1 embryonic/atrial, encoded by the myosin light chain 4 (MYL4) gene. These myosin isoforms are transiently expressed during embryonic and fetal development and disappear shortly after birth when adult fast and slow myosins become prevalent. These myosins are re-expressed during muscle regeneration and provide a specific marker of regenerating fibers in the pathologic skeletal muscle (Schiaffino et al., 2015).

Several molecules have been identified in myogenesis and have been found overexpressed in IIM muscle: DLK1, Nephilysin, Nestin, Cyclin-dependent protein kinase 5, Leukotriene B4, C1q (Andersen et al., 2009; Broccolini et al., 2006; Naito et al., 2012; Sjöberg et al., 1994; Wilczynski et al., 2000). These findings support the myogenesis involvement during the pathogenesis process of IIM.

3.1.2 Cellular alterations that impact the regeneration process

3.1.2.1 Regenerating myofibers *in vivo* and IIM muscle regeneration

In an immunohistochemical study, Baumann et al showed that MRFs were significantly up-regulated in jDM muscles with different expression in early (irregularly shaped capillaries, deposition of C5b-9 on still preserved capillaries and signs of myofiber de- and regeneration) and advanced lesions (perifascicular regions with prominent loss of capillaries, myofiber atrophy and endomysial fibrosis) (Baumann et al., 2018). The number of satellite cells labelled for Pax7 was increased in jDM damaged muscles as compared with control muscles, especially in perifascicular atrophic areas. In early lesions, myogenic cells expressing MyoD and Myogenin were moderately increased. In advanced lesions, dysregulation of the myogenic regenerative pathway was supported by the absence of MyoD in a context of increased Myogenin expression (Baumann et al., 2018). In another immunohistochemical study, Wanschitz et al found that the number of satellite cells and regenerating fibers were significantly increased in DM, PM and IBM, and the increase was proportional to the severity of muscle inflammation. In sIBM muscle, MyoD+ nuclei (identifying activated satellite cells and proliferating myoblasts) was lower, and myogenin+ nuclei (marker of differentiation) was higher in sIBM in comparison to DM and PM (Wanschitz et al., 2013). Such dysregulation of MRFs drove the authors on the consideration of muscle repair impairment.

3.1.2.2 MPCs and IIM muscle regeneration

*** IIM derived-MPCs**

Across the literature, only two studies reported analysis of MPCs derived from IIM biopsies (IIM-MPC) (Cseri et al., 2015; Hayashi et al., 1990). Starting from cell culture of a single DM patient muscle, Hayashi et al evaluated the myogenesis process. After dissection and enzymatic digestion, muscle cells were cultured without specific MPC sorting (non-available at that time), resulting in mix of various cells and myotubes. The authors found no differences between the control muscle culture and the DM muscle culture. In the second work, primary cell cultures of human MPCs derived from untreated IIM patients, treated with corticosteroids IIM patients and healthy control were compared. The authors identified an impaired proliferation and a slightly altered fusion capacity in MPCs started from untreated IIM specimens (Cseri et al., 2015). Cell proliferation was characterized by the increase in the number of myogenic nuclei normalized to the value at the 2nd day of culture (Cseri et al., 2015). Differentiation was assessed by fusion index (number of myogenic nuclei in the myotubes divided by the total number of the myogenic nuclei). Of note, the lack of MPC sorting, may introduced potential severe bias in the evaluation, with at least entanglement of other muscle-derived cell types. In addition, Cseri et al did evaluate differentiation step by using fusion index, and did not evaluate the MRF expression, which would have allowed more accurate myogenesis step deciphering.

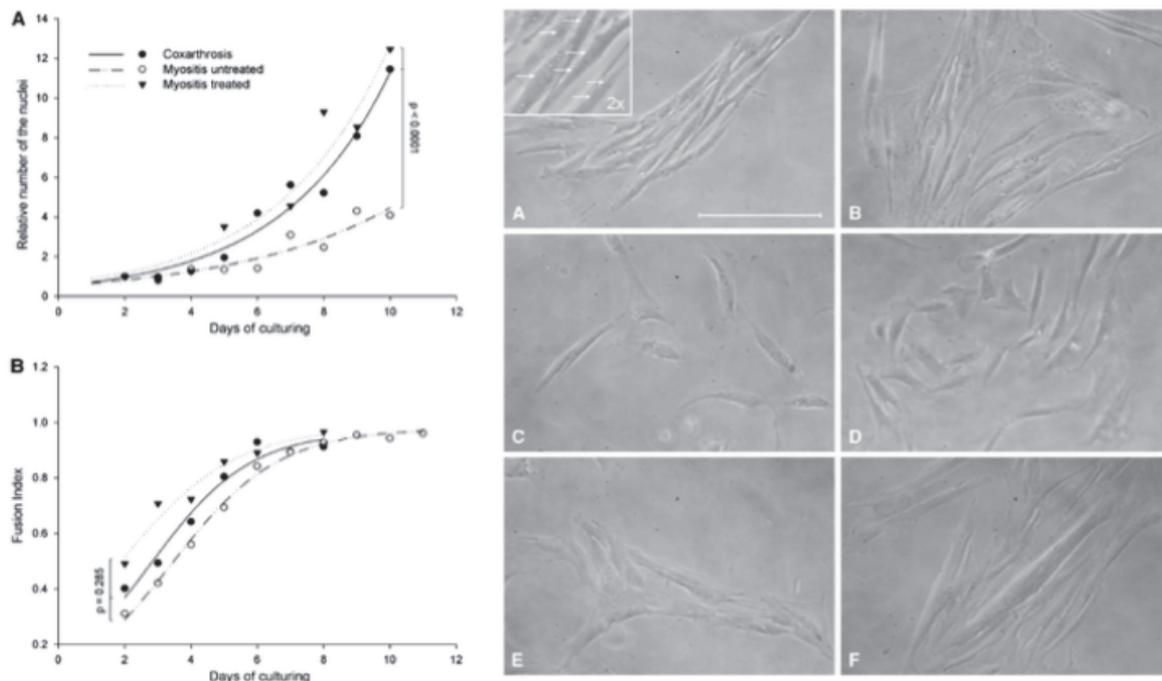


Figure 10. Left panel: Proliferation and fusion properties of myoblasts in primary cultures initiated from patients with coxarthrosis and idiopathic inflammatory myopathies (with or without corticosteroid treatment). Significance levels are displayed for separate curves versus single curve non-linear fitting. a Number of myogenic nuclei normalized to the number of myogenic nuclei on the 2nd day of culturing. Two parameter exponential curves have been fitted to the measured data. b Ratio of myogenic nuclei in multinuclear cells compared to the total number of myogenic cells. Three-parameter sigmoid curves have been fitted to the measured data. **Right panel:** Phase contrast micrographs of myoblasts and myotubes in primary culture at the 6th and 10th day of culturing initiated from patients with coxarthrosis (a, d), idiopathic inflammatory myopathy without corticosteroid treatment (b, e) and idiopathic inflammatory myopathy with previous corticosteroid treatment (c, f). Scale bar indicates 10 micron, magnification is same for all panels. On the 92 magnified insert of panel a, some myogenic nuclei are marked with arrows. (From Cseri et al 2015)

* In vitro model of IIM micro environment and consequences on myogenesis

Another approach is to recapitulate *in vitro* the *in vivo* pathogenic conditions found in IIM. As described above IFN-I is known as a crucial cytokine involved in DM pathogenesis. IFN-I are known as naturally produced negative regulators of growth that can also modify cell differentiation in multiple cell types (Hertzog et al., 1994). Regarding IFN-I and *in-vitro* myogenesis, three studies have to be considered, all evaluated human myoblasts (Fisher et al.,

1983; Ladislau et al., 2018), including one also evaluating C2C12 mice myoblast (Franzi et al., 2013).

In the model of the myogenic mouse C2C12 cell line, effects of type I or type 2 IFN on myotube formation were assessed by measuring their length, diameter and area, and the induction of ISG15 expression by myoblasts. The authors identified that IFN-I, particularly IFN- β , treatment impaired myoblast differentiation into myotubes in a dose-dependent manner with the larger dose resulting in fewer and shorter myotubes (Figure 11.) (Franzi et al., 2013).

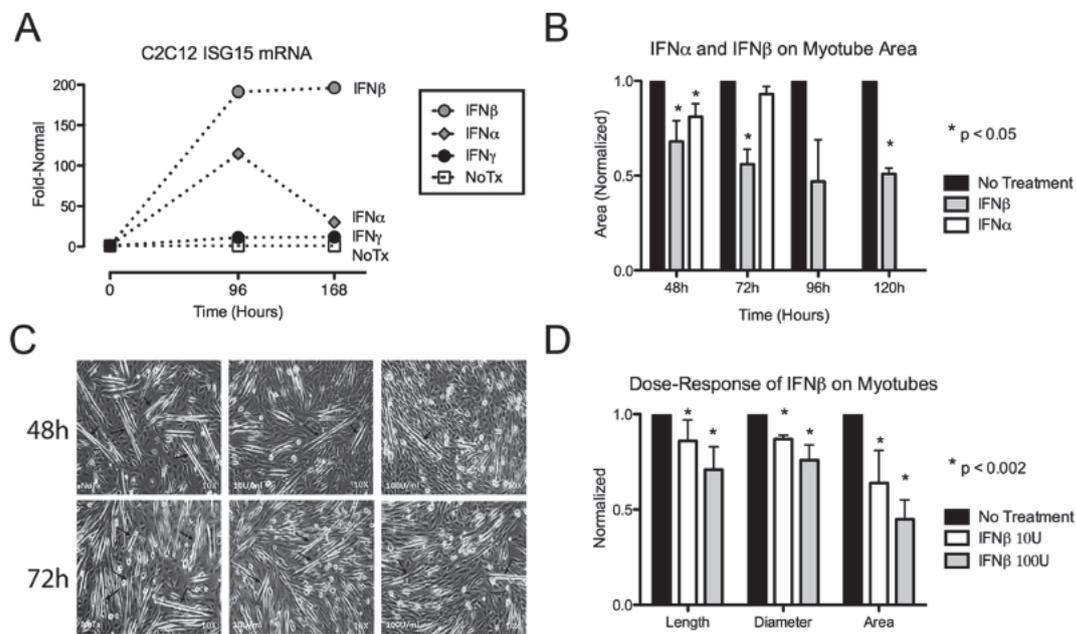


Figure 11. Effects of type 1 IFNs on mouse C2C12 and human muscle cells. (A) IFN- β results in sustained marked expression of ISG15 (196-fold increased at Day 7). (B) Sustained toxicity of IFN- β on myotube area. (C–E) Dose-dependent effects of IFN- β 10 U/ml and 100 U/ml on myotubes. (C) Dose-dependent reduction in numbers and lengths of C2C12 myotubes at 48 h and 72 h. Arrows indicate myotubes. (D) Dose-dependent reduction in C2C12 myotube length, diameter, and area at 72h. (Franzi et al., 2013)

Regarding IFN-I consequences on human myoblast myogenesis, two studies identified impairment of myotube formation (Franzi et al., 2013; Ladislau et al., 2018). Franzi et al identified dose-dependent effect of IFN- β on myotube formation (Figure 12A). Ladislau et al

performed various activation of IFN-I pathway, and identified a decrease of fusion index, surface area of myotubes and myogenin expression (Figure 12 B, C, D).

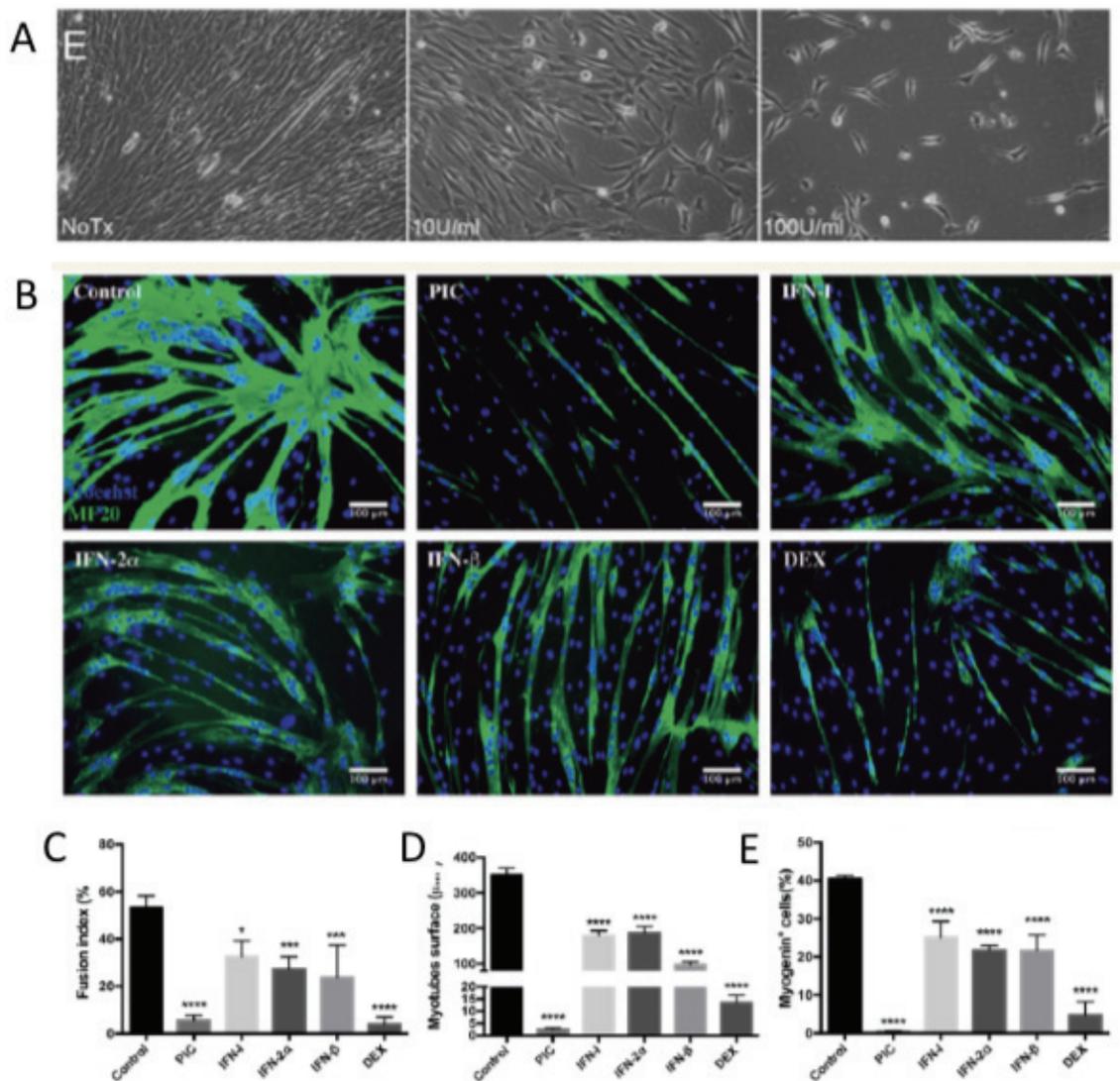


Figure 12. Effect of IFN-b on human skeletal muscle myogenesis in vitro (A) Dose-dependent effect of IFN-b on 72 h human skeletal muscle with marked inhibition of myotube formation at 100 U/ml (From Franzi et al., 2013). (B) The activation of the IFN-I pathway induces muscle atrophy in vitro. Differentiated myotubes non-stimulated (control) or stimulated with Polyinosinic-polycytidylic acid (PIC), IFN-I, IFN-2a, IFN-b or dexamethasone (DEX) were stained with anti-myosin heavy chain antibody MF20 (green) and Hoechst (blue). (C) Fusion index, (D) Myotube surface and (E) myogenin expression (Lasdilau et al., 2018).

IFN-I -induced muscle atrophy *in vitro* was associated with the upregulation of the expression of atrophy-genes (TRIM63/MURF-1 and FBXO32/atrogin). Interestingly the *in vitro* effect of IFN-I on myogenesis process was rescued by JAK inhibition (Ladislau et al., 2018).

Running counter to these findings, Fisher et al demonstrated that IFN induced an acceleration of myotube formation (Fisher et al., 1983). To discuss such discrepancies, several differences in experimental protocols have to be pointed out: IFN subtype was not defined and consisted in recombinant human leukocyte IFN produced in bacteria or from the human myeloblast cell line KG-1 in Fischer et al study, whereas in the two other works IFN was type 1 (α or β) and was IFN- α or - β (PBL Interferon Source, Piscataway, NJ) (produced by fibroblast CHO cells or in bacteria). Another crucial point is the percentage of horse serum (20% in Fisher et al *versus* 2% in Franzi et al. and 5% in Ladislau et al.), that question a potential interaction between serum and IFN.

Considering type 2 IFN (i.e. IFN- γ), a dose-dependent impairment of proliferation and differentiation of non-sorted human skeletal muscle cells have also been demonstrated (Kalovidouris et al., 1993).

3.1.2.3 Macrophages and IIM muscle regeneration

Presence of inflammatory infiltrates is one of the main IIM pathological features. Their composition is variable according to the IIM subgroup. However, one cell type is consistently found: the macrophage (Loell and Lundberg, 2011). As mentioned above, macrophages exert important supportive roles on adult myogenesis by adopting sequential inflammatory status (Juban and Chazaud, 2017).

Hayashi et al have performed a study on a single patient DM muscle compared to control non-sorted muscle cells culture. The muscle culture was secondarily exposed to DM him-self mononuclear cells collected from patient's blood by density gradient centrifugation methods. The authors observed that treatment with autologous mononuclear cells induced vacuolar degeneration of myotubes while myoblasts remained unaffected. Authors hypothesized that cell-mediated cytotoxicity may be responsible for *in vitro* and potentially *in vivo* muscle degeneration (Hayashi et al., 1990).

On another level, macrophage phenotype has been studied in anti-HMGCR-associated myopathy. This work defined macrophage as the predominant infiltrating cell type and identified prominent M2 polarization (Chung et al., 2015). Macrophage subtypes are classically defined as M1 (or pro-inflammatory macrophages) and M2 (or anti-inflammatory macrophages). This suggests that infiltrating macrophages are more likely to be involved in

muscle repair than in inflammatory respond in such cases (Chung et al., 2015). This pivotal role of macrophage cells in muscle regeneration and the consideration of a defective muscle repair in IIM call for further evaluation of these cells, and their potential role in muscle repair impairment in IIM.

3.1.2.4 Endothelial cells and IIM muscle regeneration

Capillaries and EC are known to be altered in IIM, and particularly in DM. Reduced capillary density, enlargement of capillaries, tubulo-reticular inclusion within the EC, and immunoglobulin as well as complement found at EC surfaces are common features of DM (Baumann et al., 2018; Lundberg, 2001; Pinal-Fernandez et al., 2019). This consideration questions the link between muscle regeneration impairment and EC alteration. As mentioned above, MPCs and ECs are privileged partners during muscle regeneration (Latroche et al., 2017). In an *ex vivo* study on jDM muscle biopsy, the early phase of myogenesis (defined by deposition of C5b-9 on still preserved capillaries and signs of myofiber degeneration and regeneration) was associated with EC activation, while MRF expression (MyoD, Myogenin) was altered in perifascicular regions with capillary drop out. This supported the contribution of myogenic differentiation impairment to perifascicular atrophy of muscle fibers (Baumann et al., 2018).

Lasdislau et al showed that activation of IFN-I pathway impaired EC angiogenesis *in vitro*. After IFN-I treatment, authors reported a sustained reduction in the total segment length, in the number of meshes and junctions between EC (Ladislaue et al., 2018). In a work focused on jDM, an IIM characterized by focal capillary loss in addition to the usual IIM's pathological features and enhanced IFN-I signaling, Gitiaux et al have found that jDM derived-MPCs exhibit highly angiogenic properties associated with IFN-I gene upregulation. The authors demonstrated the possibility to recapitulate the molecular pattern and the proangiogenic properties of normal MPCs by IFN-I treatment (Gitiaux et al., 2018).

3.2 Mechanisms involved in alteration of muscle regeneration in IIM

Several studies suggest that degree of muscle damage or inflammation found on IIM muscular biopsy does not correlate consistently with the clinical severity, arguing for nonimmune

pathways that may play a role in muscular impairment of myositis patients (DeVere and Bradley, 1975; Lundberg, 2001)

3.2.1 Metabolism impairment that alter myogenesis

3.2.1.1 Hypoxia and muscle regeneration in IIM

Considering the well-known vascular damage in DM and jDM, hypoxia is considered as an important part of the pathogenesis. In addition, several of the cytokines or soluble protein reported as overexpressed in IIM, notably TNF- α , IL-1 and HMGB1, can be induced by hypoxia. In a study on jDM, Preuss et al found that jDM was mainly characterized by hypoxia-driven pathology in perifascicular small fibers (numerous atrophic neonatal myosin+ fibers costained with markers of hypoxia such as VEGFA) and by macrophages expressing markers of hypoxia (Preuß et al., 2016). The RIG-I, an ISG that is overexpressed in perifascicular areas of DM muscle, has been characterized as hypoxia-inducible factor in human MPCs, which may induce local IFN-I production (De Luna et al., 2017). *In vitro*, culture of MPCs in hypoxic conditions mimicked the angiogenic signature of jDM-derived MPCs, and phenocopied their proangiogenic properties on ECs, supporting the role of hypoxia in jDM pathogenesis (Gitiaux et al., 2018).

3.2.1.2 Mitochondrial impairment and muscle regeneration in IIM

In a congress abstract, Basualto-Alarçon et al described mitochondrial dysfunction with decreased ATP-linked oxygen consumption in IIM-derived MPCs (Basualto-Alarcón et al., 2017). Through transcriptomic analysis of DM muscles from untreated patients, mitochondria-related genes (involved in mitochondria integrity and function) were identified as the most down-regulated genes (Basualto-Alarcón et al., 2017). These findings were corroborated by histochemical analyses showing irregular NADH dehydrogenase (mitochondrial respiratory complex I) and succinate dehydrogenase (SDH, complex II) activity staining, and a highly increased number of cytochrome c oxidase (COX, complex IV) in DM muscles as compared with control muscles (Meyer et al., 2017). *In situ* oxygraphy analysis showed reduced mitochondrial coupling between respiration and oxidative phosphorylation in DM muscle. This was correlated with increased ROS production, and poor aerobic capacities of DM patient

muscle. The proposed trigger of such mitochondrial alteration was IFN- β through the induction of ROS, supporting the crosstalk between immunity, mitochondrial functions and ROS production. This hypothesis was validated on an experimental autoimmune myositis, in which mitochondrial impairment, muscle weakness and inflammation were prevented by ROS scavenging (Meyer et al., 2017).

Boehler et al analyzed the epigenetic changes by methylation profiling in myotubes infected by coxsacki B and compared them to epigenetic changes in muscle samples from PM and DM patients. The authors identified that harakiri (HRK), a mitochondria-localized activator of apoptosis, was upregulated in myositis-derived MPCs. The authors found that the expression of HRK was inversely correlated with cell viability, and that HRK-induced mitochondrial dysfunction resulted in defective plasma membrane repair. Lastly, the study demonstrated that after a damage, healthy control MPCs overexpressing HRK presented an impairment in their repair capacities, supporting the hypothesis that HRK may play a role in defective muscle regeneration in IIM (Boehler et al., 2019).

In a work studying Cytochrome c oxidase deficiencies in the muscle of IIM patients (DM, PM and IBM), Chariot et al suggested that capillary loss results in tissue hypoxia and may contribute to the observed mitochondrial dysfunction (Chariot et al., 1996).

3.2.1.3 Endothelial reticulum (ER) stress and muscle regeneration in IIM

The ER has several critical roles in the folding, export, and processing of newly synthesized proteins. When there is an imbalance between the load of proteins in the ER and the cell's ability to process that load, a collection of signaling pathways that adapt cells to ER stress are turned on. The ER stress response can be provoked by a variety of pathophysiologic conditions, including ischemia, viral infections, mutations that impair protein folding, and excess accumulation of proteins in the ER (Mori, 2000). Cells self-protect against ER stress by initiating several responses. At least 4 functionally distinct responses have been identified: 1) responses involving up-regulation of the NF- κ B pathway (ER overload response), 2) up-regulation of genes encoding ER chaperone proteins such as BiP/ glucose regulated protein (Grp) 78 and Grp94, to increase protein folding activity and to prevent protein aggregation, 3) translational attenuation to reduce the load of protein synthesis and to prevent further

accumulation of unfolded proteins (unfolded protein response), and 4) cell death, which occurs when functions of the ER are severely impaired.

Activation of the ER stress response in IIM and its potential role in muscle fiber damage and dysfunction has been supported by several works. In a study evaluating muscle tissue from human myositis patients and from a MHC-I transgenic mouse model of myositis, the authors demonstrated an activation of the ER stress response. Indeed, the pathways of ER stress response, the unfolded protein response (Grp78 pathway), and the ER overload response (NF- κ B pathway) were significantly activated in muscle tissue of human myositis patients and in the mouse model (Nagaraju et al., 2005). As discussed below, engagement of Nuclear factor-kappa B (NF- κ B) pathway may then be responsible for muscle regeneration impairment. In a study investigating the ER stress-response occurring in myofibers of patients with IIM, Vitadello et al identified abnormal Grp94 overexpression, an ER chaperone involved in immunomodulation, as a characteristic of regenerating myofibers (Regenerating myofibers were identified by human embryonic My isoform labelling) on IIM muscle biopsies. This supported an ER-stress response in regenerating myofibers, which could potentially participate to defective muscular regeneration (Vitadello et al., 2010). In another study Delaunay et al showed overexpression in sIBM muscle of the ER-Bound RING Finger Protein 5 (RNF5), an ubiquitin-protein ligase implicated in the ER associated degradation misfolded proteins process. In transgenic mice over-expressing RNF5 either ubiquitously or in a muscle specific manner, regeneration coupled to myofiber degeneration regeneration was associated with altered expression of ER chaperones. A long-term muscle-specific RNF5 overexpression recapitulated the sIBM muscle impairment. Lastly, RNF5 KO mice exhibit a delay in regeneration from cardiotoxin-induced muscle damage (Delaunay et al., 2008). All that taken together support the hypothesis of RE impairment as part of the altered muscle regeneration in IIM.

3.2.2 Molecular pathways involved in impaired muscle regeneration in IIM

Normal skeletal muscle fibers do not express class MHC-I proteins. During mouse muscle regeneration induced by a local freeze injury, MHC-I is expressed by MyoD⁺ myoblasts but not by regenerating myofibers (labelled by embryonic myosin heavy chain) (Pavlath, 2002). Interestingly, overexpression of MHC-I by myofibers is a hallmark of IIM histology (van der Pas et al., 2004). MHC-I role has been hypothesized in the initiation and maintaining of muscle

damages, allowing T cell mediated cytotoxicity and presentation of muscle autoantigens by muscle fibers themselves. To evaluate the implication of MHC-I in IIM pathogenesis, and specifically with muscle repair impairment, several studies have to be considered. A model of transgenic mice with MHC-I gene overexpression in skeletal muscle fibers recapitulates the histological features of IIM with muscle cell damage and inflammatory infiltrates, arguing about the hypothesis of MHC-I involvement in IIM pathogenesis (Nagaraju et al., 2000). In another study, experimental IFN- γ overexpression in primary mouse myoblasts and in regenerating mouse muscle leads to MHC-I overexpression by regenerating myofibers, and impairment of muscle regeneration (Pavlath, 2002). In order to evaluate the direct effect of MHC-I on muscle differentiation, the same authors tested MHC-I gene transfer *in vivo* and *in vitro*, and found myogenesis impairment, notably the differentiation step (Pavlath, 2002). Lastly, the link between MHC-I overexpression and RE stress response has been discussed, as MHC-I staining in muscle fibers of myositis patients presented cell surface but also sarcoplasmic reticulum pattern of internal reactivity, demonstrating that some of the class I MHC may be retained in the ER of these fibers. The ER overload response is known to activate NF- κ B pathway, with subsequent expression of pro-inflammatory cytokine and regeneration impairment (Nagaraju et al., 2005).

NF- κ B is a transcription factor involved in both immune reactions and myogenesis. NF- κ B is composed of different subunits responsible for the so-called classical or alternative NF- κ B signaling pathways. Inhibition of NF- κ B pathway favors muscle regeneration in injured and diseased skeletal muscle. In a mouse model of the NF- κ B p65 subunit haploinsufficiency, Lu et al identified enhanced proliferation and myogenic differentiation. These results were strengthened by *in vitro* experiments in which selective pharmacological inhibition of IKK β , an upstream activator of NF- κ B, enhanced wild type MuSC differentiation into myotubes (Lu et al., 2012). Based on this, several cytokines and soluble proteins, such as TNF- α , IL1 β , IL15, HMGB1 may be implicated in the deleterious activation of this pathway, and their expression are in turn potentially induced by NF- κ B pathway, creating a positive feedback loop. The expression of TNF- α , known as an activator of NF- κ B pathway, was shown increased in IIM muscle biopsies (De Bleecker et al., 1999; Efthimiou, 2006; Kuru et al., 2003). The role of TNF- α in NF- κ B up regulation in muscle was confirmed *in vitro* by an increase in NF- κ B nuclei translocation, traducing the pathway activation, after treatment of MPCs by TNF- α (Chevrel et al., 2005). This supports the hypothesis of impairment of muscle regeneration by TNF α ,

through activation of NF- κ B. Interestingly, *in vitro* treatment of MPCs by TNF- α also induced MHC-I expression by myoblasts (Chevrel et al., 2005).

The High-Mobility Group Box 1 protein (HMGB1) is an alarmin, responsible for immune response stimulation, and has been identified as having multiple effects on skeletal muscle regeneration (De Mori et al., 2007). Through immunohistochemical investigation, Cseri et al identified a decrease of HMGB1 positive myogenic nuclei in IIM muscle fibers as compared with control. HMGB1 has been involved in the TLR4 pathway in PM and DM and may play a role in muscle fatigue of the patients by inducing MHC-I expression on muscle fibers and reducing the sarcoplasmic reticulum Ca²⁺ release (Cseri et al., 2015).

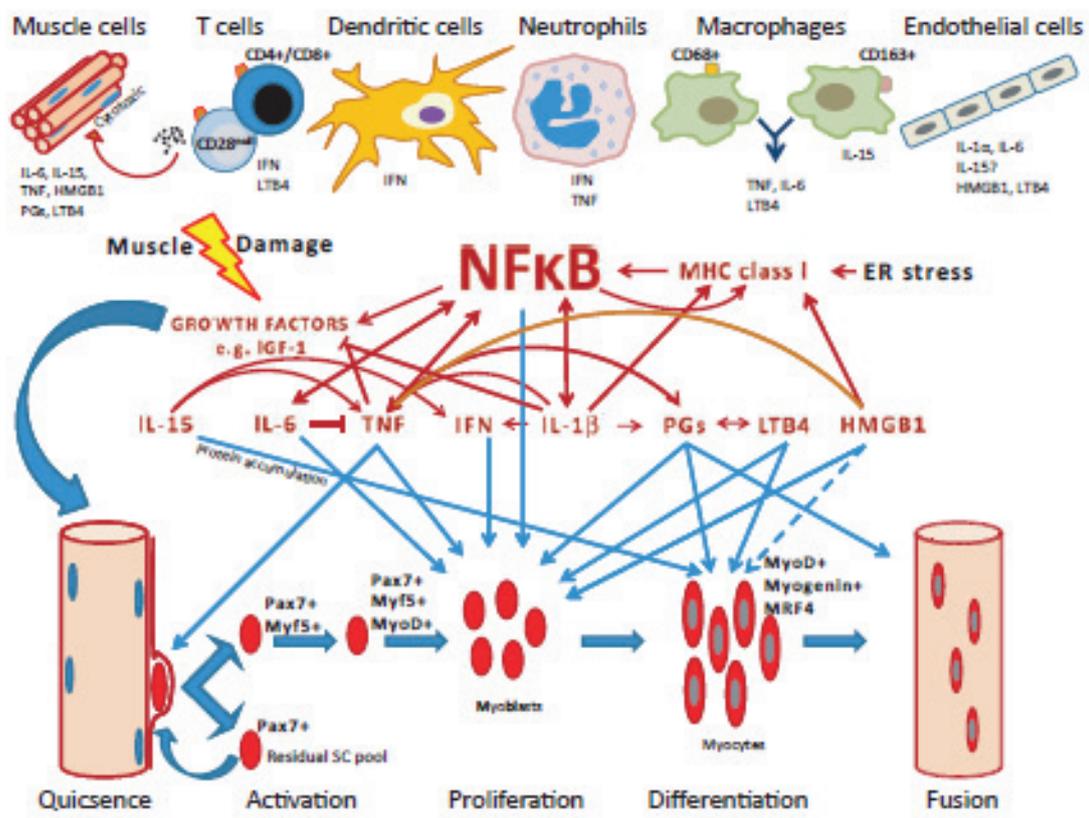


Figure 13. A schematic diagram illustrating the positive effects on muscle fiber regeneration and remodeling of molecules that might have a dual role in muscle tissue both promoting inflammation and affecting skeletal muscle fiber regeneration and remodeling in myositis patients. The key molecules can influence each other (red arrows) as well as the different phases of skeletal muscle regeneration; the focus in this diagram is on the less frequently described positive effects (blue arrows) rather than the established negative effects. The broken blue arrow indicates inhibiting effects on muscle fiber differentiation. Upon skeletal muscle fiber

damage, an inflammatory/immune response precedes myogenesis. The local immune response in muscle tissue has a direct effect on satellite cell activation, proliferation, differentiation or fusion. This local immune response might be disturbed (e.g. by immunosuppressive treatment), thus potentially influencing the functional restoration of fibers injured in various ways. Therefore, suppressing the immune system or blocking single cytokines or receptors might disturb skeletal muscle regeneration. SC, satellite cells. (From Loell et al., 2011)

3.3 Muscle regeneration as an actor of IIM pathogenesis

3.3.1 Muscle regeneration, autoantigens and autoimmunity

The involvement of regenerating muscle cells in IIM pathogenesis has been proposed by Casciola-Rosen et al (Casciola-Rosen et al., 2005). In this work, analysis of IIM muscle biopsies identified that regenerating myofibers (defined by expression of CD56 and neonatal myosin heavy chain) were overexpressing myositis specific autoantigens (catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), and histidyl-tRNA-synthetase (HRS)). Overexpressions were reproduced in undifferentiated MPCs in vitro. This has been confirmed in another study of muscle regeneration in acutely injured mice (Sciorati et al., 2015). Through these observations, authors pointed the fact that the regeneration process may take part in the chronicization of IIM. Indeed, increased autoantigen expression in myogenic cells accomplishing repair of firstly damaged myofibers could lead to an amplification cycle by long-lasting presence of the “triggers” of adapted and innate immune responses (Suber et al., 2008). Therefore, MPCs could be both victim and victimizer in the pathogenesis leading to abnormal immune response and chronic muscle inflammation. In addition, DM are known to be associated with cancer in a substantial proportion of cases. Several studies have identified co-expression of similar autoantigen expression by regenerating myofibers and various adenocarcinoma (Casciola-Rosen et al., 2005; Suber et al., 2008), adding by the way a third player in this autoimmune reaction enhancement.

Studying immunopathologic processes of IIM in experimental murine models, Sciorati et al found that in the presence of TLR/adjuvant signals and underlying muscle injury, apoptotic myogenic precursors expressing high levels of autoantigen could recapitulate IIM features (autoantibody formation and inflammatory infiltrates within muscle) (Sciorati et al., 2015).

3.3.2 Autoantigens and myogenesis process

TIF-1 γ , an autoantigen identified in DM, also known as a transcription factor involved in various tissues, is upregulated in nuclei of myofibers expressing markers of regeneration in DM muscle biopsies (Mohassel et al., 2015). Silencing *in vitro* this protein in cultured C2C12 myoblasts shows accelerated myosin heavy chain (MyHC) expression (but not myogenin expression) and fusion, supporting the hypothesis of a link between autoantibody/autoantigen expression and factor involved in myogenesis (Mohassel et al., 2015). The same team has also evaluated Mi-2, a chromatin remodeler, known to be another myositis autoantigen. Mi-2 was overexpressed in myofibers expressing regeneration markers in DM muscle biopsies, and was upregulated during post-injury muscle regeneration in the mouse. Silencing of Mi-2 by RNA interference *in vitro* resulted in accelerated differentiation of MuSCs. Globally, these results suggest that high levels of Mi-2 expression in muscle biopsy tissue from patients with DM may reflect the presence of incompletely differentiated muscle cells (Mammen et al., 2009).

3.3.3 IFN and regeneration process

Tournadre et al demonstrated that *in vitro*, MPCs activated by PIC, the ligand of TLR-3, overexpress MHC-1 during myogenesis, thereby reproducing the overexpression observed in DM histology. MHC-1 overexpression was decreased after IFN- β blockade. This work also demonstrated that after activation by PIC and IFN- γ , MPCs secretes IFN- β through activation of the TLR3 pathway (Tournadre et al., 2012).

3.4 Muscle weakness and muscle repair impairment as a result of drug side effect

Another element that may play a role in ineffective muscle repair of IIM patients is the impact of the immunosuppressive drugs used in DM treatment. Notably, glucocorticoids, which are the cornerstone treatment of several IIM, are also known to have catabolic effect on muscle mass, and direct and indirect inhibitory effects on myogenesis (Hanaoka et al., 2012).

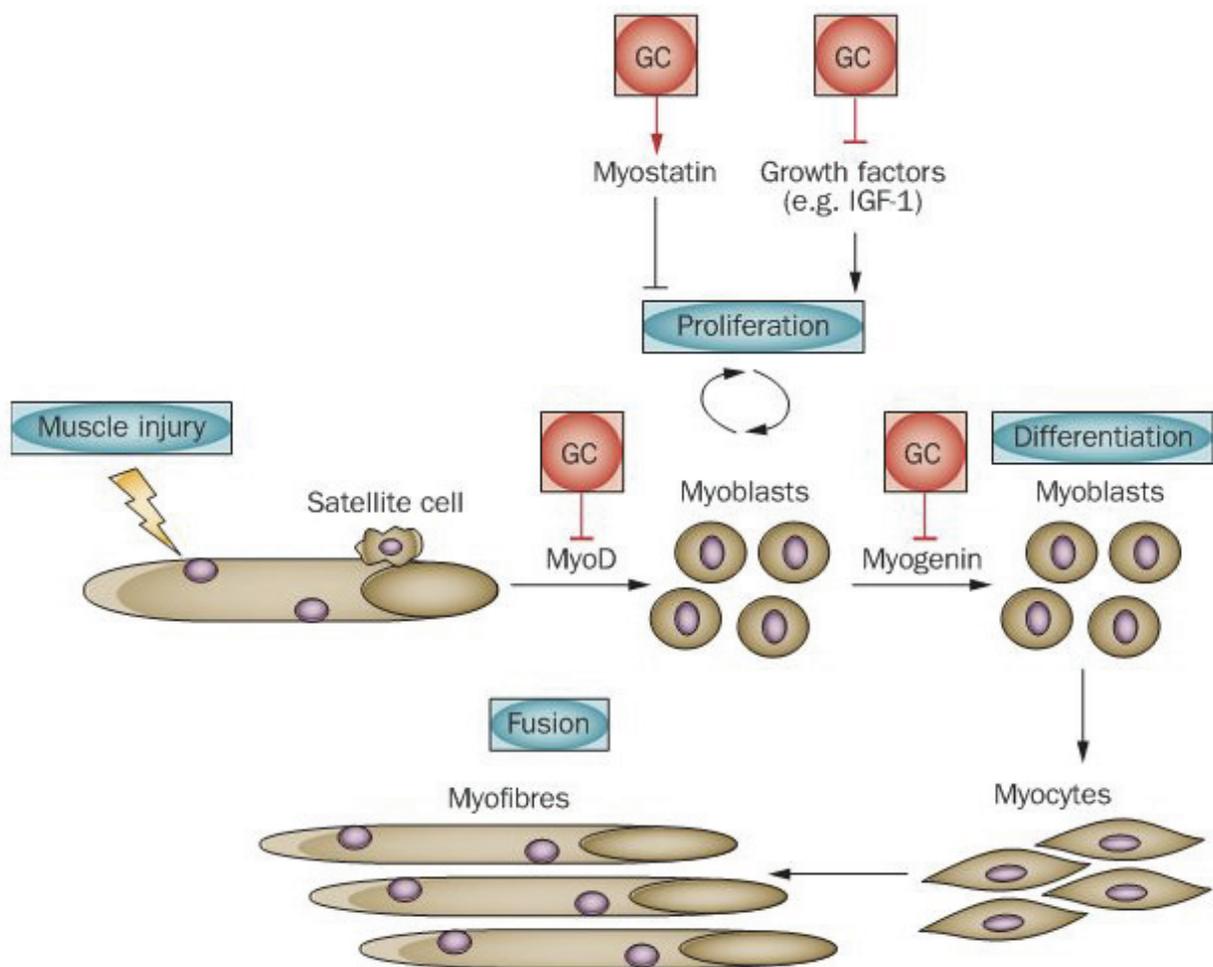


Figure 14. The effects of glucocorticoids on postnatal skeletal muscle regeneration. Following injury, muscle stem cells (satellite cells), located under the basal lamina of muscle fibers are activated to proliferate and differentiate. Satellite cells, identifiable by the expression of the transcription factor Pax7, fuse to form new myofibers. Muscle regeneration is regulated by a family of myogenic transcription factors (MyoD, myogenin, Myf5 and MRF4), growth factors (FGFs, HGFs, and IGF-1), and myostatin, a negative regulation of differentiation in myoblasts. Glucocorticoids affect muscle regeneration by upregulating expression of myostatin, while inhibiting myogenic transcription factors (myogenin and MyoD) and growth factors that promote satellite cell and myoblast differentiation into myocytes. (From Hanoaka et al., 2012)

Glucocorticoids inhibit muscle regeneration by decreasing myogenic cell proliferation through proteasome-dependent degradation of MyoD in C2C12 myoblasts (Sun et al., 2008). In addition, glucocorticoids differentially regulate degradation of MyoD and its negative regulator Id1 by N-terminal ubiquitination to promote muscle protein catabolism (Sun et al., 2008). In vitro, glucocorticoids also alter differentiation by downregulating myogenin expression (Schakman et al., 2009).

Interestingly, glucocorticoids are also known to play a role on modulation of essential interactions between macrophages and MPCs during muscle regeneration (Desgeorges et al., 2019).

Analyzing muscle biopsy of PM and DM patients, Sugiura et al observed that muscle cells expressed HGF) receptor c-met, and that HGF was expressed by inflammatory infiltrates (Sugiura et al., 2010). This study pointed out the fact that corticosteroids decreased HGF production by myoblasts, and consecutively downregulated expression of myogenic-related genes. This argues for a direct detrimental effect of corticosteroids on skeletal muscle regeneration.

As illustrated in this introduction, more and more is known about muscle regeneration during IIM. Today's understanding allows to define skeletal muscle regeneration and the adult myogenesis process as one of the important points in IIM pathogenesis. Nevertheless, persistent grey areas, notably regarding cellular and molecular pathway deserve further deciphering, using in vitro and in vitro studies. Better understanding of such element may open the door of future therapeutic approaches, and please for adaptation of already in use armamentarium for such diseases.

SPECIFIC AIMS OF THE STUDY

Muscle regeneration is well understood in physiological conditions. After muscle injury, regeneration leads to the complete restoration of myofibers. This is made possible thanks to myogenic precursor cells (MPCs) that activate upon injury, and then undergo myogenesis (proliferation, differentiation and eventually fusion to form new myofibers). This process depends on various intracellular or extracellular factors that are provided by the cell environment. Cultures of MPCs have been used for a long time to understand cellular and molecular mechanisms of myogenesis.

Idiopathic Inflammatory myopathies (IIM) are a heterogeneous group of autoimmune diseases leading to a severe muscle impairment. During the last decades, advances in understanding IIM clinicopathology are noteworthy. This progress led to a better delineation of IIM subgroups, with organ specificity and adapted therapeutic approaches (Allenbach et al., 2018; Mammen et al., 2020; Mariampillai et al., 2018).

Despite the increased knowledge, grey areas persist, amongst which the residual weakness undergone by a significant proportion of patients despite maximal medical care (Bronner et al., 2006; Clarke et al., 1995; Lundberg, 2001). Considering the muscle damage, regeneration process is supposed to repair *ad integrum* the tissue after the use of immunosuppressive drugs to control the dysimmune reaction, which is not the case. A defect in muscle regeneration process has been hypothesized by several authors. The pathological mechanisms involving muscle fiber damages as well as MPCs for muscle repair are not fully understood.

To better understand the status and the role of muscle regeneration in IIM outcome, namely muscle weakness and fatigue, we evaluated the centerpiece of muscle regeneration: the myogenic precursor cell (MPC). To this end, DM appeared of particular interest, first because it is one of the most frequent IIM. DM affects young as well as adult patients and is characterized by a strong IFN-I signature that is correlated with the severity of the disease (Greenberg et al., 2012; Huard et al., 2017). Moreover, pathological features are either common to other IIM features: inflammatory infiltrates, MHC-I sarcolemmal overexpression, or specific to the disease, including microangiopathy (resulting in capillary drop out), perifascicular atrophy, and perifascicular regions containing regenerating myofibers (expressing NCAM/CD56) (Dalakas, 2015). This points out the involvement of regenerating myofibers in the disease process, as well as endothelial cells, a privileged partner of MPCs. Involvement of the IFN-I pathway in DM pathogenesis is supported by clinical case reports,

immunohistological studies and transcriptomic analysis (Baechler et al., 2011; Dietrich et al., 2000; Greenberg et al., 2005).

Our working hypothesis is that the chronic outcome of DM is potentially due to inefficient skeletal muscle regeneration process and we specifically asked if such process would be due to altered myogenic properties of MPCs, which ones and how? The understanding of muscle regeneration in IIM is crucial to develop new therapeutic strategies to limit muscle damage.

The specific objectives of my PhD work were:

- *Clinical/histological aim.* DM is highly heterogeneous regarding the variation of pathological muscle changes and the clinical outcome. The clinical/histological part of the program was to delineate pathological severity gradation and to correlate it with simple clinical-biological features (muscle weakness, maximal creatine kinase level, auto antibody identified).

Muscle biopsy alterations were assessed after establishment of a DM severity score, inspired by the consensus jDM score tool (Varsani 2015). DM severity score was usable once cases met two of the three main pathological criteria: inflammatory infiltrates, capillary dropout (identified with CD31 staining) and MHC-I overexpression. This classification helped to define two pathological subgroups in DM: mild and severe. Evaluation of MPC pool was performed *ex-vivo* by Pax7 immunostaining on DM and HC muscle biopsies.

- *Cellular aim.* This part included the analysis of myogenic properties of DM derived-MPCs *in vitro*, by studying global myogenesis process and then deciphering each step of this process: proliferation, commitment into differentiation and fusion in multinucleated myotubes. This work evaluated the intrinsic myogenic properties of patient derived-MPCs without external additional treatment, since I worked with purified MPCs. DM myogenic cells were compared with myogenic cells from healthy age- and sex-matched subjects to gain further insights in the pathogenesis of the disease. Next, the microscopic aspect of DM derived-MPCs in culture drove us to further investigate MPC fate (cell cycle and senescence).

- *Molecular aim.* In this part, the functional and cellular analysis was completed by a molecular investigation of the IFN-I pathway that is known to be involved in DM pathogenesis. This analysis consisted in assessing IFN-I signature of DM patients and HC derived-MPCs, myogenesis rescuing experiments with IFN-I receptor blocker and JAK/STAT pathway

inhibitor (Ruxolitinib). An IFN-I impairment of the normal myogenesis process was also set up in order to determine the possibility to phenocopy DM derived-MPC impairment.

Considering the results obtained during the first two years of this work, the PHD program has been extended to several other IIM, notably, IBM, IMNM and ASS. Preliminary results are presented here.

RESULTS

1.1. Clinical research: Study of a cohort of focal myositis

A first translational research work focused of the delineation of FM in order to further defined this particular IIM entity: **Gallay L et al, Focal Myositis: New insights on diagnosis and pathology, Neurology 2018**

Focal myositis

New insights on diagnosis and pathology

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Abstract

Objective

To better define in a cohort study the clinical and pathologic features of focal myositis (FM).

Methods

With the use of the usual clinicopathologic definition, each confirmed case of FM in the Lyon University Hospital's myopathologic database between 2000 and 2016 was retrieved. Clinical, pathologic, imaging, serologic, and therapeutic data were collected. When data were missing but feasible, appropriate pathologic analyses were performed.

Results

Of the 924 patients included in the database, 37 (4%) had confirmed FM (14 female, 23 male patients). The main symptoms were pain ($n = 30$, 81%), muscular mass ($n = 16$, 43%), erythema at the site of FM ($n = 12$, 32%), and fever ($n = 9$, 24%). Serum creatine kinase was normal in most patients (81%); serum immune abnormalities were frequent (inflammatory syndrome in sera [39%], dysglobulinemia [52%], and anti-nuclear antibody positivity [29%]). In addition to confirming previously reported findings, pathologic analyses found significant rates of vasculitis (68%) and fasciitis (73%). Here, FM appeared frequently to be associated with other diseases such as immune-mediated inflammatory disease (IMID; 32%), neoplasia (24%), and radiculopathy (11%). Regarding outcomes, 64% of the cases had received immunosuppressive drugs, and the relapse rate was 41%.

Conclusion

The present study suggests that FM is not as innocuous as previously believed, particularly considering the associated disorders. Notably, patients with FM should receive careful IMID and neoplasia screening.

CME Course

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Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

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Glossary

CREST = calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia; **FM** = focal myositis; **FMSS** = FM simple score; **HPS** = hematoxylin-phloxine-saffron; **IMID** = immune-mediated inflammatory disease; **MHC-I** = major histocompatibility complex class I; **NADI** = no associated disorders identified.

First described in 1973,¹ focal myositis (FM) is a rare disease defined by the association of an isolated inflammatory pseudotumor usually restricted to 1 skeletal muscle, focalized abnormalities on MRI or EMG, and myositis on pathologic examination. The reported symptoms can include local pain, although the prevalence of pain varies considerably in the literature. In most cases, FM is reported as having a spontaneous resolution after a typically acute to subacute clinical course.¹⁻⁴ Approximately 250 cases have been described in the literature,⁵ but mostly in the form of case reports or small series. Only 1 study has been performed in a large cohort and with a precise pathologic description of FM and clearly defined objectives.² This limited literature might explain the paucity of knowledge on the clinical features and therapeutic management of FM. Occasionally, FM has been described in association with disorders such as radiculopathies, as well as infectious and autoimmune diseases.⁶⁻⁹ The prevalence of these associations remains unknown.

The nonspecificity of pathologic features (marked variations in fiber size and hypertrophy, inflammatory infiltrates composed mainly of macrophages and T cells, regenerating fibers and fibrosis, circumscription to 1 muscle) also raises questions as to the pathophysiology and nosology of the disease. Given the heterogeneity of its clinical phenotype, it remains unclear whether FM should be considered a syndrome associated with other conditions or an individual disease. Thus, in the present work, we sought to further define nosologic, clinicopathologic, and disease-association aspects of FM via an analysis of a large cohort of patients with myositis.

Methods

Case selection and data collection

Screening of the myopathologic database of 924 cases of myositis diagnosed between 2000 and August 2016 at the University Hospital of Lyon (France) was performed to retrieve all cases of FM. To facilitate this, we built an FM simple score (FMSS) that synthesized hallmarks from the literature (figure 1). Eligibility criteria were FMSS ≥ 2 and adequate material for the intended studies. We excluded cases of FMSS < 2 and ocular myositis during Grave disease, known as a distinct entity.¹⁰ For all cases, medical, biological, morphologic, and electrophysiologic data were retrieved. Macroscopic (mass location, monocompartment or multicompartment involvement, and lateralization) and microscopic (hematoxylin-phloxine-saffron [HPS] sections) pathologic specimens were analyzed for all cases. When

missing but feasible, appropriate pathologic analyses were performed.

Standard protocol approvals, registrations, and patient consent

Human biological samples and associated data were obtained from Cardiobiotec Biobank (CRB-HCL Hospices Civils de Lyon BB-0033-00046). All tissue samples were obtained according to French Legislation (specifically, written informed consent was obtained from patients for all samples). Cardiobiotec is authorized by the French Ministry of Social Affairs and Health (DC2015-2566) with transfer authorization (AC 2015-2576). The Biobank database is registered in the national data protection commission (Commission nationale de l'informatique et des libertés).

Immunohistochemistry studies

A standardized protocol was used for biopsy analyses. Fresh muscle specimens were divided into 2 fragments, 1 fixed in 10% neutral buffered formalin and the other frozen in isopentane. The formalin-fixed fragments were embedded in paraffin, and morphologic (HPS) and immunohistochemistry studies were performed with anti-CD3, CD4, CD8, CD20, CD68, major histocompatibility complex class I (MHC-I), and CSb9 antibodies (detailed methods are given in supplementary methods [e-Methods, links.lww.com/WNL/A248] and table e-1 [links.lww.com/WNL/A250]). Immunohistochemistry results were scored semiquantitatively.

Statistical analyses

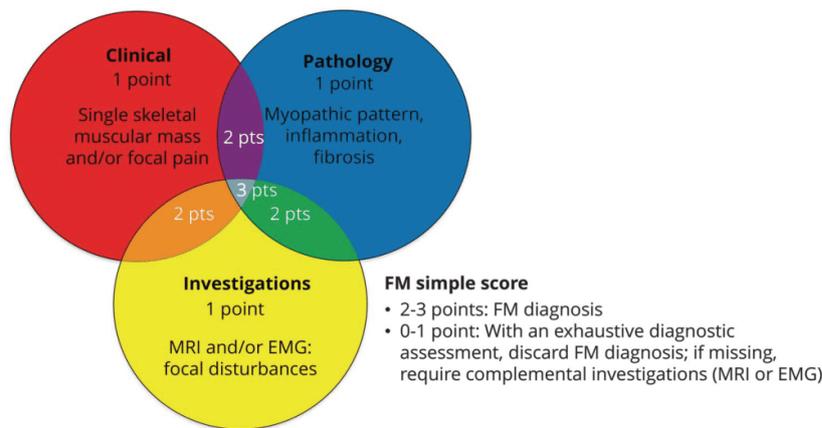
Quantitative variables were summarized by mean, SD, median, quartiles 1 and 3, minimum, and maximum, while qualitative variables were described by number and percentage. Variables identified a priori were compared between each of the associated-diagnosis groups and the others. Comparisons between groups were performed with nonparametric tests (Wilcoxon test and Fisher test for quantitative and qualitative variables, respectively). Age was compared between FM subsets by use of the nonparametric Kruskal-Wallis test. Differences with values of $p < 5\%$ were considered significant. In case of significance, nonparametric post hoc tests were performed to identify which groups led the overall significance of the difference.¹¹ Analyses were performed with R software (software 3.0.2, R Computing, Vienna, Austria, 2013).¹²

Results

Patient characteristics

Among the 924 screened myositis patients, 45 cases of FM (5%) were identified, among which 37, with confirmed

Figure 1 Calculating the FMSS



One point is given when the clinical criterion is fulfilled (presence of a single muscle mass found on clinical examination and/or focal pain reported by the patient); 1 point is given when the pathology criterion is fulfilled (muscle biopsy revealing inflammatory cells along with other myopathic features, i.e., basophilic regenerating fibers, degenerating fibers, myophagocytosis, and/or fibrosis); and 1 point is given when the MRI/EMG criterion is fulfilled (MRI consistent with a single focus of myositis, i.e., gadolinium-enhancing focus on T1-weighted images, a hyperintense focus on T2-weighted imaging, or myogenic features on EMG that are restricted to a single muscle). Patients with 2 or 3 points were classified as having focal myositis (FM). FMSS = FM simple score.

diagnosis and adequate amount and quality of material, were retained for further study (figure 2). There were 23 male and 14 female patients. Patient ages ranged from 18 to 80 years; the mean age was 44 years (median 43 years). The delay from FM occurrence to diagnosis ranged from a few days to 5 years; the mean delay was 16 months.

Associated disorders

FM occurred in association with other associated disorders: immune-mediated inflammatory disease (IMID; n = 12, 32%), neoplasia (n = 9, 24%), radiculopathies (n = 4, 11%), and trauma (n = 2, 5%; figure 3A). IMID included Behçet disease (n = 5), various inflammatory rheumatic diseases (Sjögren syndrome, lupus, CREST syndrome [calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia] and Still disease; n = 5), autoimmune thyroiditis (1 patient), and uveitis and psoriasis (1 patient). Neoplasms were solid cancers in 7 cases (bladder n = 2, thyroid n = 2, lung = 1, prostate n = 1, and adenocarcinoma of unknown primary site n = 1) and hemopathies in 2. These neoplasia were diagnosed synchronously (n = 3), secondarily in the following year (n = 5), or within 4 years (n = 1). At the time of the study, 10 cases of FM (27%) had no associated disease despite extended clinical exploration and therefore were included in the no associated disorders identified (NADI) subgroup. Among the subsets of FM-associated disease, median ages were significantly different (global $p = 0.014$), and individuals in the NADI subgroup were younger than those in the radiculopathy ($p = 0.0023$) and those in the neoplasia subgroups ($p = 0.0017$, figure 3B).

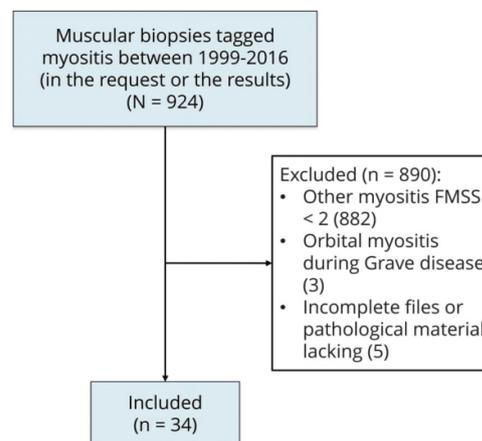
Clinical characteristics

Clinically, a circumscribed intramuscular mass within 1 muscle was reported in 62% (n = 23), and multi-monofocal myositis (involving a maximum of 3 muscles) within the same or different compartments was reported in 38% (n = 14). FM

occurred in the lower limb in 70% (n = 26), upper limb in 22% (n = 8), and head or neck in 8% (pharyngeal muscles n = 2, mentalis muscle n = 1).

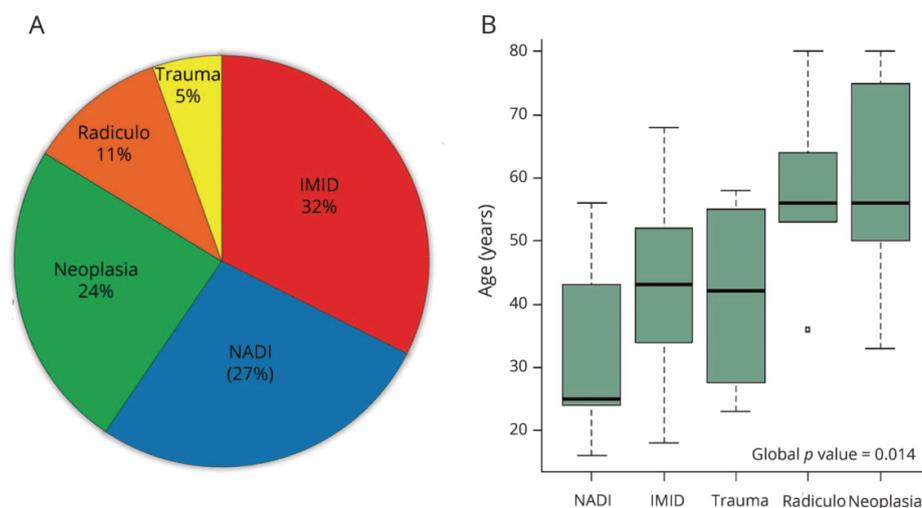
Four symptoms were frequently reported: pain (n = 30, 81%), muscular mass (n = 16, 43%), erythema at the site of FM (n = 12, 32%; mainly in the NADI and neoplasia groups), and fever (n = 9, 24%; more frequent in the IMID group, $p = 0.004$, table e-2, links.lww.com/WNL/A250). No muscle weakness was reported, but impotence was observed in 3 cases, in each case in association with severe pain.

Figure 2 Study flowchart



From the complete myopathologic database of the Lyon University Hospital between 1999 and 2016, those with other myositis were excluded, followed by those with incomplete files or for whom pathological material was lacking and subsequently those with orbital focal myositis. FMSS = focal myositis simple score.

Figure 3 Disorders associated with focal myositis



(A) Distribution of associated disorders in and (B) box plot representation of age for each subset (thick black line indicates median). IMID = immune-mediated inflammatory disease; NADI = no associated disorders identified; Radiculo = radiculopathy.

Investigational findings

Creatine kinase level was elevated, ranging from 300 to 1,500, in 6 of 32 cases (19%), including 2 who had radiculopathy. Elevated erythrocyte sedimentation rate or C-reactive protein was found in 39% of cases (12 of 31), especially those with IMID (45%), NADI (50%), or neoplasia (56%). Anti-nuclear antibody was positive in 29% (8 of 28) in the IMID and neoplasia subsets (42% and 29%, respectively). Serum protein electrophoresis identified dysglobulinemia in 52% (11 of 21; 10 with polyclonal and 1 with monoclonal gammopathy), including 5 patients with IMID and 3 with neoplasia. *Borrelia burgdorferi* tests performed were all negative ($n = 8$).

Electrophysiologic studies were abnormal in 80% (16 of 20), showing essentially focal myogenic features ($n = 12$) or neurogenic patterns with radiculopathy (L5 or S1 roots $n = 4$). MRI found focal inflammation (hyperintensity on T1/T2-weighted images, fast-short tau inversion recovery images, and gadolinium contrast-enhanced images in 93%, 25 of 27), and fascia impairment was found in a 26% (7 of 27 cases) (figure e-1, A and B, links.lww.com/WNL/A249). PET/CT was performed for 6 patients, leading to a diagnosis of cancer ($n = 3$ of 6, 50%; an example is given in figure e-1, C and D).

Microscopic findings

All patients had muscle biopsies; 3 biopsies were of inadequate quality and were excluded from the analysis. HPS was performed on these 34 biopsies; there were varying degrees of missing data for other immunohistochemistry analyses.

Myopathic changes (basophilic regenerating fibers, degenerating fibers, myophagocytosis) were found in 56% of cases, and

internalized nuclei were seen in 71%. FM associated with IMID had less necrosis ($p = 0.006$, figure 4). Conversely, FM associated with neoplasia ($p = 0.043$) and radiculopathy ($p = 0.046$) had more necrosis (table e-2, <http://links.lww.com/WNL/A250>).

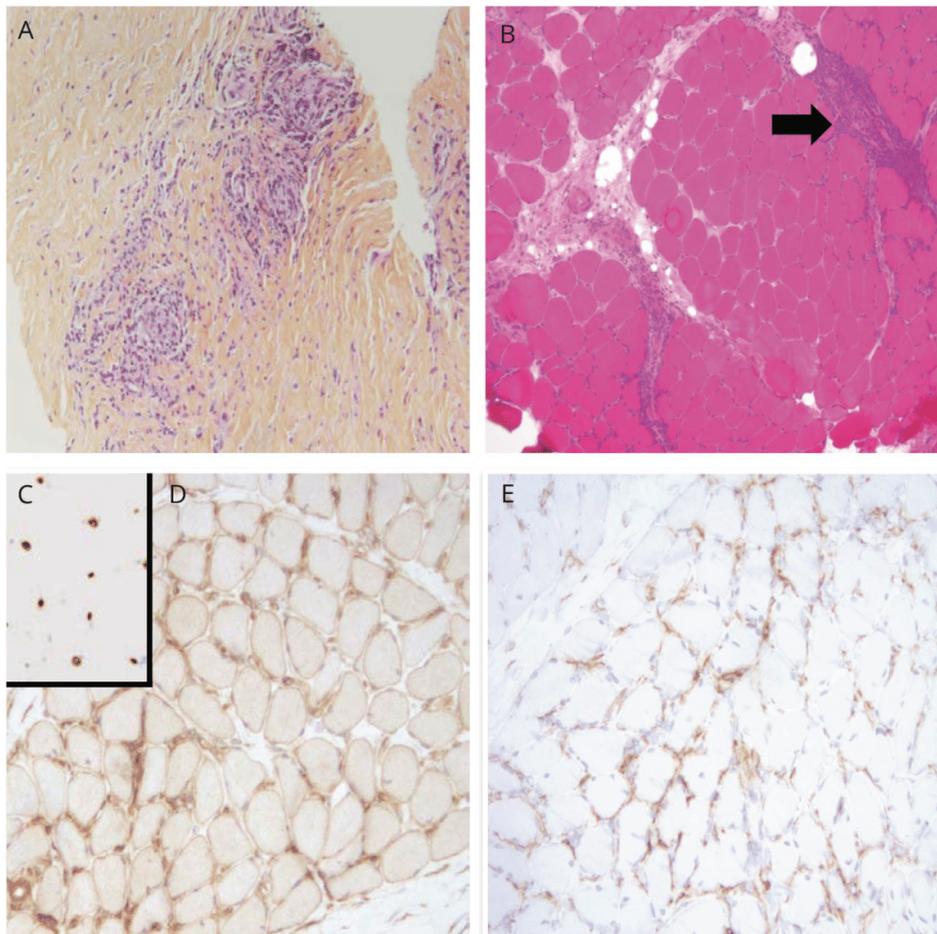
Dystrophic muscle modifications were as follows: atrophic fibers (91%), variation in myofiber size (88%), endomysial and/or perimysial fibrosis (59%), hypertrophic fibers (56%), and adipose involution (26%).

Fibrosis was common and appeared less frequently in FM associated with IMID than in FM without IMID ($p = 0.005$, figure 4 and table e-2, links.lww.com/WNL/A250).

Inflammatory infiltrates were observed in all 34 cases (100%) and in fasciae (fasciitis) in 19 of 26 cases with data available (73%; figure 4A). Muscular infiltrates were perivascular (80%), perimysial (76%), endomysial (59%), and often mixed. In addition, vasculitis (defined by alteration and immune cell infiltration of the vessel wall) was observed in 68% of biopsies (figure 4B); there was 1 case of necrotizing vasculitis ($n = 1$). The occurrence of vasculitis varied depending on the FM-associated disease: neoplasia (75%), IMID (77%), NADI (67%), trauma (50%), and radiculopathy (20%). Vasculitis ($p = 0.029$) and fasciitis ($p = 0.047$) were less frequent in those with radiculopathy than in those without (table e-2, links.lww.com/WNL/A250).

A neurogenic pattern of pyknotic nuclear clumps ($n = 12$, 35%), grouped atrophy ($n = 5$, 15%), and fiber type grouping ($n = 5$, 15%) seemed particularly associated with radiculopathy.

Figure 4 Pathologic abnormalities on muscular biopsies of patients with FM



Examples of fasciitis (A, hematoxylin-phloxine-saffron [HPS] $\times 20$) and vasculitis (B, arrow, HPS $\times 20$) are shown. Immunohistochemistry: compared to normal control muscle (C, $\times 40$) an abnormal major histocompatibility complex class I expression on the sarcolemma of muscle fibers was found (D, $\times 40$). C5b9 immunohistochemistry was also abnormal with myofibers perimembranous staining (E, $\times 20$). FM = focal myositis.

Lymphocytes, plasma cells, and macrophages were present in the infiltrates of all patients, although in variable proportions. T cells (CD3+) showed marked staining in all cases and were the most prominent cells in the infiltrates. Among the 24 biopsies with data available, all had CD4+ T cells, and 56% CD8+ T cells. Macrophages (CD68+) were present in 93% (26 of 28), usually scattered within muscles. B cells (CD20+) were detected in 81% (22 of 27) of biopsies, mostly in perivascular clusters.

MHC-I was overexpressed on the sarcolemma of muscular fibers (figure 4D) in 88% of biopsies (21 of 24), with variable intensity. C5b9 staining was observed in 21 of 24 biopsies (88%) and showed various staining profiles: mild punctiform endomysial deposits (71%), membranous staining (50%; figure 4E), and endomysial intracapillary microthrombi (4%).

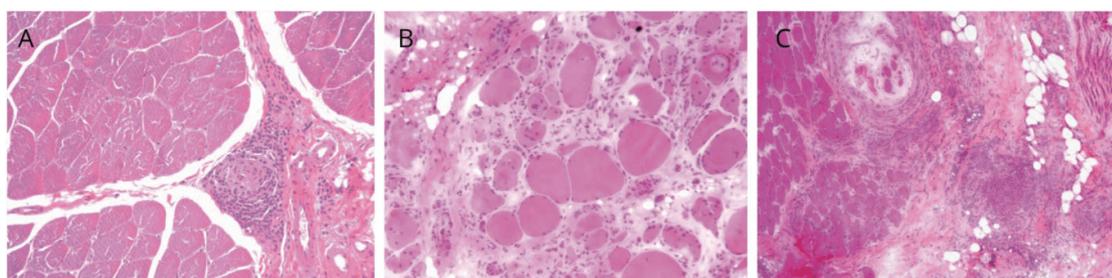
Among the 5 different clinical subsets of FM identified in the present study (NADI, IMID, radiculopathy, trauma, and neoplasia), 3 presented significant pathologic variations associated with the underlying associated disease (figure 5).

Of note, histopathologic alterations were heterogeneously distributed, with markedly pathologic muscle fascicles often neighboring less affected fascicles.

Outcome and treatment requirement

Twenty-four patients (64%) received single or combined immunosuppressive treatment with glucocorticoids ($n = 23$), colchicine ($n = 6$), methotrexate ($n = 4$), azathioprine ($n = 3$), intravenous immunoglobulin ($n = 2$), or cyclophosphamide and tumor necrosis factor blockers ($n = 1$ each). Over a mean follow-up of 6.4 years, 15 patients relapsed (41%), among

Figure 5 Variation of pathologic pattern according to FM-associated diseases (hematoxylin-phloxine-saffron)



(A) Focal myositis (FM) associated with IMID $\times 40$ (myositis pattern with muscle fibers relatively unscathed, with vasculitis and fasciitis). (B) FM associated with radiculopathy $\times 40$ (myositis with grouping of atrophic fibers, nuclear centralization, fibrosis, and necrosis). (C) FM associated with neoplasia $\times 20$ (myositis with important disturbances of the muscle, fibrosis, vasculitis, and necrosis).

whom 3 had a single self-limited episode. Cases of relapse were FM associated with IMID ($n = 6$) and neoplasia ($n = 3$). One patient benefited from surgical removal of the affected muscle tissue.

Discussion

While FM has long been considered a harmless disorder, the results of the present study highlight the potential for serious disease, particularly regarding the associated conditions.

The possibility of bias herein cannot be excluded because the study was performed retrospectively, in a single center, and on a small number of patients. Prospective studies would be of great value but also difficult to perform because of the rarity of FM, as reflected in the literature.^{1-3,13,14} Although comprising only 37 patients, the cohort is sizable for the study of clinical, biological, morphologic, and pathologic aspects of FM and permits the identification of trends. The single-center nature of the study can be considered advantageous in that it ensured a homogeneous evaluation of the entire FM cohort. Data collection was exhaustive and standardized, and the techniques for clinical and pathologic analyses were performed by a single person.

In this cohort, FM was frequently associated with IMID, neoplasia, radiculopathy, and trauma. Elsewhere, FM triggered by radiculopathy-associated denervation has been described in case reports.^{6,7,15,16} With regard to IMID-associated FM, Behçet disease and inflammatory rheumatic diseases were the most prevalent IMID, and while 13 similar cases of Behçet disease have been reported,^{8,17,18} other inflammatory rheumatic diseases have rarely been reported (psoriasis,¹⁹ Sjögren syndrome,²⁰ and lupus⁹). The high rate of neoplasia reported here contrasts with the literature in that solid cancers or hemopathies have been reported in only 4 cases.²¹⁻²⁴ Concerning the NADI subset, extended monitoring would be of interest to ensure that no other associated disorders such as neoplasia or IMID appear in the following years.

The demographic characteristics of the population studied are consistent with the literature. There was variation of the median age according to associated disorders; those with NADI and IMID were younger than those with radiculopathy- and neoplasia-associated FM. This suggests that the prognosis will be different according to the age at FM occurrence, and it emphasizes the need to follow up patients with FM regularly. In contrast to some previous reports, FM was frequently painful in this report (81% vs 27% in the main cohort study),² and fever was not typically found other than in FM cases associated with IMID. As previously reported, the anatomic distribution in the present study was mainly focal; multi-monofocal disease in a single or 2 different compartments was less common.¹⁴ For these cases, multi-monofocal FM was more frequent among those with radiculopathy (50%) than among those without radiculopathy (22%). It is of note that the multi-monofocal cases here were confirmed as such, not global myositis, via assessment of their clinical and radiologic evolution. In addition, with a mean of 6.4 years of follow-up, no progression to diffuse inflammatory myositis was observed. This finding contradicts some earlier studies²⁵⁻²⁷ and argues in favor of the concept of FM being a separate entity.

Most patients had normal serum creatine kinase, but this was markedly elevated in 1 case, potentially explained by the concomitant use of statins.^{28,29} In contrast to previous reports,^{2,3} plasma immunologic abnormalities were not uncommon in the present cohort. Inflammatory syndromes, autoantibodies, and gammopathies appeared to be more common in FMs associated with IMID and neoplasia. Considered together, these results raise the question of the possibility of immune dysregulation in FM pathogenesis.

Electrophysiologic and morphologic investigations were essential for FM diagnosis study, and their key contribution was to demonstrate the absence of abnormalities outside the FM location. When associated with radiculopathy, there was a related nerve root location for the affected muscle, coherent

with nerve injury topography. In accordance with the literature,^{14,30} MRI scanning found the usual focal inflammation pattern but also fascial impairment in a quarter of cases. Only 1 MRI was normal, but it had been performed late in the history of the disease. We also note that half of the performed PET/CT scans revealed malignant lesions. Considering the frequency of neoplasia occurrence in the present cohort, the contribution of PET/CT in the initial evaluation of FM should be assessed.³¹

Here, the muscle biopsies remained a major tool to reach the diagnosis of FM. However, 3 biopsies were less informative than the others: 1 had not been performed in the most affected muscle, and 2 had been performed under full-dose immunosuppressive treatment. This emphasizes the need to perform biopsy before therapeutic initiation and in pathologic muscle as identified by MRI.

Beyond confirming the histologic findings of previous reports, the pathologic analyses demonstrated a frequent occurrence of fasciitis (73%) and vasculitis (68%) and the prevalence of MHC-I and C5b9 staining. Immunohistochemistry analysis found predominant T cells (CD3+) expressing CD4+ more than CD8+, as already described.² However, B cells (CD20+) and macrophages (CD68+) were encountered mainly in FM cases associated with IMID and neoplasia, which favors immunologic disturbances; although CD20 cells showed a clustered staining pattern, no germinal centers were identified. Perimembranous MHC-I staining with heterogeneous distribution was frequently observed. It is often observed in overlap syndrome and differs from the perifascicular pattern seen in dermatomyositis or the diffuse pattern described in inclusion body myositis.^{32–36} C5b9 staining is a very important criterion in the pathologic diagnosis of dermatomyositis or necrotizing myopathies,^{37,38} whereas its usefulness in FM remains unknown. C5b9 positivity was observed in 88% of cases, with various kinds of staining that did not show any association with associated diseases.

Indeed, FM associated with IMID appears as myositis pattern with muscle fibers relatively unscathed, with less necrotic fibers ($p = 0.006$) and fibrosis ($p = 0.005$), and with frequent fasciitis and vasculitis. FM associated with radiculopathy appeared as a myositis with frequent grouping of atrophic fibers, hypertrophic fibers, nuclear centralization, fibrosis, and significantly more necrotic fibers ($p = 0.046$). FM associated with neoplasia appeared as a myositis with important disturbances of the muscle with fibrosis, vasculitis, fasciitis, and significantly more necrosis ($p = 0.043$). NADI and trauma-associated FM did not show differences compared to the usual pathologic FM definition.

The results suggest that immunologic mechanisms may play a prominent role in the disease process, with or without coexisting neoplasm. The high occurrence of vasculitis, particularly in cases of FM associated with IMID (100%) and cancer (75%), raises the question of this mechanism in the

pathogenesis of FM, thus raising the possibility to consider FM as a single organ vasculitis in some cases. In the literature, single organ vasculitis in muscle has rarely been reported but has been described as being similar to localized periarteritis nodosa.^{39,40} Of the 23 patients having vasculitis in the current study, only 1 had necrotic vasculitis, which undermines this concept. Moreover, here, vasculitis lesions appeared more like the microangiopathy described in dermatomyositis. Radiculopathy and trauma were also frequently associated, suggesting another potential clinicopathologic pathway.

Furthermore, an interesting aside is that delay to diagnosis of FM varied from a few days to 5 years, which is possibly attributable to the very low prevalence of this disease. Except in cases of FM associated with trauma, relapses were observed more frequently than has been reported in previous studies.^{1,2} The retrospective nature of the present study limits what can be said about treatment. It was observed that immunosuppressive therapy was prescribed in more than half of the cases, which runs counter to the literature. Treated cases were very painful relapsing or persistent FM (data not shown). Despite the absence of consensus or Cochrane review, glucocorticoids were used as the first-line treatment and seemed to be efficient. In some cases of clinical worsening, steroid dependence, or relapse, other immunosuppressive drugs such as azathioprine or cyclophosphamide were prescribed. Conversely, methotrexate, the most widely used treatment for myositis, was used in only 4 of the 24 treated FM cases.^{41–44} These results underline the necessity of prospective studies to clarify how FM should be managed.

The findings of the present study lead us to consider FM as a clearly identifiable entity, even if the question of whether FM is a syndrome or a disease unto itself remains to be answered. We describe new clinical and pathologic features to further define FM, enabling us to identify different subsets. Identification of this entity, and its associated disorders, appear important for patient management, as it may be a harbinger of malignancy.

Author contributions

Laure Gally: designed the study, collected data, analysis and interpretation of the data, wrote draft. Arnaud Hot and Philippe Petiot: interpretation of the data, revising the manuscript for intellectual content. Françoise Thivolet-Bejui: revising the manuscript for intellectual content. Delphine Maucourt-Boulch: statistical analyses, helped to interpret results. Nathalie Streichenberger: designed the study, interpretation of the data, revising the manuscript for intellectual content.

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Disclosure

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1.2 Translational research: evaluation of regeneration during DM

The second part of the results focus on the study of pathogenesis of dermatomyositis, and more specifically on the regeneration of muscle during the muscular damage. Personal work at the laboratory bench and analysis are completed, and presented below in the form of an original article that aim to be submitted to Annals of the Rheumatic Disorder by the end of year 2020.

Impairment of adult myogenesis by muscle stem cells in dermatomyositis

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Abstract

Dermatomyositis (DM) is the most frequent Idiopathic inflammatory myopathies (IIM), occurring during child and adulthood, and is potentially life-threatening. Diagnosis is based on the association of muscle weakness, specific histological pattern on muscle biopsy and auto-antibodies in the serum. Etiopathogenesis remains largely unknown. In DM, the necrosis/regeneration balance is detrimental to muscle integrity, with a chronic course and persistent weakness for some patients. Nevertheless, the capacity of muscle regeneration has been barely studied. In normal injured muscle, regeneration is achieved by myogenic precursor cells (MPCs) that undergo myogenesis process to build new myofibers. The aim of our project was to evaluate DM-derived MPC capacity to undertake myogenesis.

Methods.

DM (severe [n=5] and mild [n=3]), and healthy controls (HC [n=5], sex and age paired) were selected in accordance with the 239th ENMC criteria 2020. Supplemental investigation was performed on juvenile DM (JDM) derived-MPCs (n=3) with paired HC (n=3). Myogenesis was explored in vitro using specific MPC culture set up for the evaluation of proliferation (by incorporation of a thymidine analogue into DNA during cell cycle), differentiation (by myogenin expression evaluation) and fusion (by quantification of the number of nuclei incorporated into myotubes). Similar methods were used to evaluate the impact of interferon blockers (JAK / STAT inhibitor and IFN-I receptor antagonist (IFNAR)) on proliferation. Senescence was analysed by flow cytometry with fluorogenic substrate of SA- β Galactosidase, a senescence-specific marker. Quantitative analysis was performed using Image J software, and statistical analysis was performed by mean comparison (t test or ANOVA).

Results.

Analysis of MPC proliferation indicates that DM-derived MPCs exhibited a significantly lower proliferation than HC-derived MPCs ($p=0.0020$). Terminal myogenic differentiation was strongly impaired in severe DM-derived MPCs while less altered in mild-DM-derived cells ($p=0.001$). MPCs fusion was significantly altered in DM-derived MPCs with a dramatic decreased observed in severe DM and to a lesser degree in mild DM ($p<0.0001$ for each). These results were reproduced for other JDM, with altered myogenesis. Our work thus focused on the first step of myogenesis: proliferation. Through cellular and molecular investigations, we found an increased senescence in DM derived-MPCs ($p=0.0020$), and a potential involvement of interferon type I in myogenesis impairment. Indeed, DM derived-MPC proliferation was improved when culture was performed in the presence of IFN-I blockers.

Conclusion.

These results show that skeletal muscle regeneration is impaired in JDM and DM due to altered capacities of MPCs to proliferate, to differentiate and to fuse. These results support the hypothesis of intrinsic alterations of MPC properties in DM, potentially linked with interferon pathway. This may explain in part the muscle alterations and muscle weakness that are observed in patients.

Introduction

Dermatomyositis (DM), the most frequent subgroup of Idiopathic Inflammatory Myopathies, is an acquired disease that combines clinic-pathological muscle and skin impairments of various severity. This disease occurs in adults and children and is designated juvenile DM (JDM) in the latter case. Notwithstanding the use of various immunosuppressive drugs, the disease follows a chronic course in most cases with rare outcomes of recovery of the initial strength (Bronner et al., 2006; Clarke et al., 1995). Identification of myositis specific auto-antibodies promoted studies focused on the classical humoral pathogenetic concept. However, DM pathogenesis remains partially understood, and seems to combine many factors, including genetic background, environmental factors, metabolism dysfunction as well as adaptative and innate immunity dysregulations (Ceribelli et al., 2016). Through histologic and transcriptomic analysis of DM muscle samples, Interferon type 1 (IFN-I) and, to a lesser extent, Interferon type 2 were identified as key elements in DM (Arshanapalli et al., 2015; De Paepe, 2015; Greenberg et al., 2005; Muskardin and Niewold, 2018; Pinal-Fernandez and Mammen, 2018), and their expression level was reported to correlate with the disease activity (Huard et al., 2017). Interestingly, histological observation of muscle biopsies shows both patterns of muscle damage and regeneration of various extent in all DM patients, indicating that muscle regeneration is an active process during DM (Winter and Bornemann, 1999).

Post-injury muscle regeneration is a well-understood process leading to the *ad integrum* restoration of damaged myofibers. This is made possible thanks to myogenic precursor cells (MPCs) that activate upon injury, and then undergo adult myogenesis (expansion/proliferation, differentiation and eventually fusion to form new myofibers) (Musarò, 2014). This process is tightly and intrinsically controlled by the sequential expression of specific transcription factors and is also regulated by extracellular cues provided by the cell environment.

Our working hypothesis was that the chronic outcome of DM is potentially due to alteration of the skeletal muscle regeneration process itself. The present study aimed at analyzing the myogenic properties of MPCs issued from DM patients, and to investigate a possible role of IFN-I in that process.

Patients, material and methods

Case selection. DM patients were identified through screening of the MYOLYON database (Hospices Civils de Lyon, France) that identifies Idiopathic Inflammatory Myopathies in accordance with the EULAR/ACR 2017 or 239th ENMC criteria 2020 (Mammen et al., 2020). Eight DM patients with available clinical data, histological samples, frozen muscle samples and myogenic precursor cells (MPCs) were selected. Exclusion criterion was administration of immunosuppressive drugs to the patient before muscle biopsy. MPCs from patients and healthy controls (HC) paired on sex and age were obtained from the Hospices Civils de Lyon cell bank (DM studies: Cardiobiotec CRB-HCLAC 2013-1867); JDM studies: Cochin Hospital Cell Bank, Paris, agreement n°DC-2009-944). For DM patients, corresponding HC biopsy samples were obtained from surgical waste for patients undergoing programmed knee replacement (n = 5). Three additional JDM and their paired HC derived-MPCs were also analyzed. Details about juvenile DM (JDM) patients have been previously described (Gitiaux et al., 2017).

Histology and immunohistology. Histological staining and immunohistochemistry of deltoid or quadriceps muscle biopsy cryostat sections (7 μ m) included haematoxylin and eosin staining (HE), immunohistochemistry for endothelial cells (anti-CD31 M0823, 1/20, Dako), regenerating myofibers (anti-CD56, NCL-CD56-1B6, 1/100, Novocastra), human MHC class I (anti-HLA-ABC, R7000, 1/ 4000, Dako) and the membrane attack complex (anti-C5b-9, M0777, 1/50, Dako). Slides were scanned using a Zeiss Axio Observer Z1 microscope. All biopsy samples were blindly reviewed for clinical data, using the validated scoring tool for muscle biopsy evaluation in DM Patients (table 1). Visualization and quantification of myogenic progenitor cells (MPC) was labelled after fixation by paraformaldehyde 2%, and blocking buffer (0.01% Triton, 2% Fetal Bovine Serum (FBS)) with primary antibodies for Pax7 (clone Pax7; 1:50; Hybridoma Bank 4ea 3/29/18 266ug/ml; anti Mouse) with overnight incubation in wet conditions at 4°C, and secondary anti Cy3 antibody Jackson ImmunoResearch code 715-165-150 lot 127319; 1:200; Donkey anti-mouse) with 45 minutes incubation at room temperature. Slides were scanned using a (Axio Observer Time lapse Zeiss) and pictures were processed to full two-dimensional reconstruction of the section with MetaMorph 7 software (Scan-Slide option). Image analyses were performed using homemade ImageJ routines (<http://imagej.nih.gov/ij/>). Quantification of Pax7 positive cells (corresponding to nuclei fluorescence)

was evaluated on the global fasciculus of muscle, and also by comparison of per fasciculus area (2 first rows of per fasciculus cells) and endomysial area.

MPC Culture. MPCs were isolated after a first passage using antiCD56 coated magnetic beads to obtain pure myogenic cell cultures as described previously (Abou-Khalil et al., 2013; Gitiaux et al., 2018) and cultured for maximum of 3 passages in complete medium (Skeletal Muscle Cell Growth Medium Promocell 23060 supplemented with Penicillin/Streptomycin (P/S) 1%, Mix C-39365 and FBS 10%). Cell purity was checked by flow cytometry (BD FACS Canto II) using anti-CD56 antibodies (anti-CD56 APC (BD Pharmingen ref 555518) and isotypic control (IgG1 APC (BD Pharmingen ref 555751).

Myogenesis assay. Analysis of proliferation, differentiation and fusion was performed for jDM- and DM-derived MPCs using step-specific culture conditions and immunofluorescence (IF) analyses. For proliferation analysis, MPCs were seeded in 2 well-chambers (Lab-Tek, Nunc) at 3000 cells/cm² in complete medium and proliferation was assessed 48 h later through the incorporation of EdU 10 μM for 6 h (kit EdU Click-it Alexa Fluor™ 488, Invitrogen). Cells were rinsed with PBS, fixed with formaldehyde 4% for 15 min, rinsed twice, permeabilized with Triton 0.5% and EdU was revealed according to the manufacturer's instructions. Cells were briefly incubated in Hoechst 33342 (Sigma-Aldrich 14533) for nuclei visualization. After a last rinse, slides were mounted using Fluoromount (Interchim) and kept at 4°C protected from light until microscopy examination. For each sample, 10 pictures were recorded using a AxioImager Z1 Zeiss microscope and proliferation index was defined as the number of EdU positive nuclei over the total number of nuclei.

For assessment of differentiation, MPCs were first cultivated for 2 days in complete medium, then cells were trypsinized and seeded at 1000/cm² in complete medium for 4 hours in 2 well-chambers (Lab-Tek), before its replacement by differentiation medium (SMGM Promocell 23060 supplemented with P/S 1 % and FBS 0.5%) for 3 additional days of culture. Differentiation was analyzed after nuclei immunolabeling for myogenin. Cells were rinsed with PBS, fixed with formaldehyde 4% for 15 min, rinsed twice, permeabilized with Triton 0.5% and rinsed twice with PBS. Double immunofluorescence labeling was performed using Myogenin antibody 1:50 (mouse BD Pharmingen 556358) to label nuclei of myoblasts in

differentiation and Desmin antibody 1:200 (rabbit Abcam ab32362) to label the shape of the cells (through labeling of intermediate filaments) for 2 h at 37°C or overnight at 4°C, protected from light. Plates were then rinsed 3 times with PBS and incubated with the secondary antibodies for 45 min at 37°C and were rinsed with PBS three times. One of the secondary antibodies being biotinylated, plates were incubated with Streptavidine DTAF 1:200 for 20 min at 37°C. After 3 rinses, plates were briefly incubated in Hoechst 33342. After a last rinse, plates were mounted on coverslips with Fluoromount and kept at 4°C protected from light. For each sample, 10 pictures were recorded using a microscope (AxioImager Z1 Zeiss) and differentiation index was defined as the number of Myogenin positive nuclei among Desmin positive cells over the total number of nuclei in Desmin positive cells.

For fusion assessment, MPCs were seeded in T75 culture flasks (ThermoFisher) at 500 cells/cm² in complete medium for 4 h before changing medium to differentiation medium. After 7 days of culture, fusion was induced after differentiated cells were seeded in 8 well-chambers (Lab-Tek) at 40,000 cells/cm² in differentiation medium. Then, cells were rinsed with PBS, fixed with formaldehyde 4% for 15 min, rinsed twice, permeabilized with Triton 0.5% and rinsed twice with PBS. Immunofluorescence was performed with Desmin antibody 1:200 (rabbit Abcam ab32362) for 2 h at 37°C or overnight at 4°C, protected from light. After that, plates were rinsed 2 times with PBS and incubated with the secondary antibody for 45 min at 37°C. After 3 rinses, plates were briefly incubated in Hoechst 33342. After a last rinse, plates were mounted on coverslips with Fluoromount and kept at 4°C protected from light. For each sample, 10 pictures were recorded

In vitro IFN-I pathway modulation

For IFN-I pathway activation assay, MPCs derived from healthy muscle were seeded in 2 well-chambers (Lab-Tek, Nunc) at 3000 cells/cm² in complete medium. After 24 h, medium was replaced by Advanced RPMI 1640 (ThermoFisher 12633012) medium + P/S 1% + Glutamax 4mM + 0.5% FBS , with addition of human IFN-β-1a (PBL assay 11415-1, 105 units) at 3 different concentrations: 0, 10, 100 and 1000 U/ml. The change in FBS concentration was performed accordingly to previous reports in order to avoid high FBS concentration interaction with the effects of IFN-I (Franzi et al., 2013; Ladislau et al., 2018). After 48 h of culture, EdU was added and 6 h later, immunofluorescence assay was performed as described above.

For IFN-I pathway inhibition assay, MPCs were seeded in 2 well-chambers (Lab-Tek, Nunc) at 3000 cells/cm² in complete medium in the presence of ruxolitinib (inhibitor of JAK/STAT pathway) (INCB018424, CAS number: 1092939-17-7, Invivogen tlr-rux) or anti-human interferon receptor (PBL assay science, Anti-Human Interferon Alpha/Beta Receptor Chain 2, Clone MMHAR-2 (MAb), Catalog No: 21385-1). After 48 h of proliferation assay culture, EdU was added and 6 h later, immunofluorescence assay was performed as described above.

IFN signature. The expression of 6 Interferon Sensitive Genes (ISGs) was evaluated according to Pescarmona et al, 2019: SIGLEC1 (sialic acid binding Ig like lectin 1), IFI27 (interferon alpha inducible protein 27), IFI44L (Interferon induced protein 44 like), IFIT1 (interferon induced protein with tetratricopeptide repeats 1), ISG15 (interferon-stimulated gene 15) and RSAD2 (radical S-adenosyl methionine domain containing 2). Total RNA extraction was performed on growing MPCs, and collected using a kit nucleospin RNA XS (740990.50 (MN)) according to the manufacturer recommendations. The extraction kit included an individual DNase treatment. Total RNA was diluted in 30 µL RNase free water and concentration was quantified by spectrophotometry using a Nanodrop 2000 (thermoscientific). The first strand of cDNA was synthesized from 1 µg of total RNA using a Transcriptase inverse SuperScript™ II (18064071 (Invitrogen)). Nanostring (NanoString Technologies) procedure hybridized 200 ng of RNA to the probes (a reporter probe and a capture probe) to evaluate level of transcripts of each ISG. Count number obtained for the 6 ISGs were normalized by the geometric mean of 3 housekeeping genes count numbers (β-Actin, HPRT1 (hypoxanthine phosphoribosyltransferase 1) and POLR2A (RNA polymerase II subunit A)) as well as the negative and positive controls values using nSolver software. The relative expression of each ISG was determined for each normalized ISG expression divided by the median normalized expression of each ISG from a control group. Finally, the median of these 6 ISG relative expression was used to calculate the IFN score. The threshold was previously defined at 2.3.(Pescarmona et al., 2019)

Cell cycle analysis. MPCs were seeded in T75 flask at 3000 cells/cm² in complete medium. After 4 days of culture, cells were harvested, washed and incubated with Hoechst 33342 at a final concentration of 10 µg/ml then Pyronin Y was added at a final concentration of 2.5 µg/ml, as previously described (Abou-Khalil et al., 2013). Control cells were obtained after treatment

with RNase and DNase. Cells were analysed by flow cytometry (BD FACS Canto II), and Diva software was used to determine G0, G1/S and G2/M phases.

Senescence analysis. Senescence was measured by flow cytometry using a protocol adapted from Debacq-Chaniaux et al. (Debacq-Chainiaux et al., 2009) evaluating the SA- β gal activity, a enzyme with lysosomal origin. MPCs were seeded in T75 flask at 3000 cells/cm² in complete medium. After 4 days of culture, cells were pretreated with bafilomycin A1 for 1 h to induce lysosomal alkalization. followed by the use of 5-dodecanoylaminofluorescein di- β -D-galactopyranoside (C12FDG) (33 μ M), a fluorogenic substrate for β gal activity, for 1h-incubation time. Cells were washed, harvested, resuspended in ice-cold PBS and immediately analyzed using a BD FACS Canto II cytometer.

Data and statistical analysis

Results were obtained from at least 3 patients and were analyzed using t-test or ANOVA (for 3 or more samples) and a p-value below 0.05 was considered as statistically significant.

Results

Case selection and scoring

Eight DM patients were selected for the study. DM diagnosis was confirmed after histology analysis if at least 2 of the 3 following criteria were present: inflammatory infiltrates (identified on HE staining), capillary dropout (identified on CD31 staining) and MHC-I overexpression. DM severity score was set up using a four-domain analysis, adapted from Varsani et al (score instructions are available in Suppl tab. 1) (Varsani et al., 2015). This severity score delineated 2 distinct histological subgroups: severe and mild DM (respectively sDM and mDM), with a severity score <8 for mDM and >12 for sDM, resulting in 8 selected patients (mDM n=3 and sDM n=5) (**Table 1**). Moreover, a simplified clinical correlation (muscle weakness, immune status, CK) was made. Apart for one case, CK level combined with muscle weakness was consistent with the mild or severe status of DM patients (**Table 2**).

Histological and immunohistological analyses were performed to know if severity score in DM could correlate with the degree of muscle damages regarding myofibers, vessels and inflammatory status (**Figure 1**). Muscle biopsies from mDM patients showed moderate manifestations of dermatomyositis such as presence of perivascular inflammatory infiltrates and limited perifascicular atrophy on HE staining, diffuse Major Histocompatibility complex-1 (MHC-1) sarcolemmal expression, focal capillary dropout (CD31 staining), and sparse myofibers undergoing regeneration (CD56 staining). In contrast, sDM exhibited important myofiber damages featuring heterogeneity in myofiber size, severe perifascicular atrophy, necrosis, and myophagocytosis, abundant inflammatory infiltrates, fibrosis, increased perifascicular MHC-1 expression, frequent capillary dropout associated with atrophic areas and strong CD56 expression in perifascicular myofibers. Interestingly, CD56 expression in damaged tissue suggested that DM patient muscles undergo both myofiber damage and muscle regeneration that may be inefficient despite the accumulation of CD56⁺ cells. This led us to investigate *in vitro* myogenic properties of MPCs from the same patients.

We investigated MPC numbers in HC and DM muscle biopsies by analyzing the expression of Pax7, which specifies both quiescent and expanding satellite cells within the CD56⁺ cell population (Seale et al., 2000). In comparison to HC, the density of Pax7-positive cells appeared increased in sDM muscle sections (**Figure 2**). More precisely, the relative number of Pax7-positive cells to the number of myofibers increased approximately 2-fold in perifascicular areas

of sDM (where muscle regeneration is observed) as compared with HC muscle ($p=0.0005$) (**Figure 2**). Interestingly, no difference could be observed between intrafascicular areas from sDM and from HC (**Figure 2**). This result shows a specific and apparent expansion of satellite cell population in the area of active disease in DM.

Myogenic properties of MPCs in DM

After activation, satellite cells follow a well-defined process leading to muscle regeneration including proliferation, commitment and differentiation into myocytes and finally myocyte fusion to form multinucleated myotubes that prefigure the myofibers (**Figure 3A**). This process can be recapitulated *in vitro*, which allows the assessment of proliferation (EdU incorporation), differentiation (expression of the myogenic differentiation transcription factor myogenin) and fusion (multinucleated cells) in cultures initiated with purified MPCs. Here, MPCs derived from muscle biopsy samples obtained from healthy controls, mDM, and sDM were CD56⁺ cells were sorted, resulting in purity ranging from 82.8% to 98.0%. Cells were culture in growing medium, during which their proliferation was assessed, then switched to differentiation, to evaluate their differentiation and fusion capacities. The percentage of EdU⁺ cells was significantly decreased of about 17.2 and 12.9 % in both DM groups ($p=0.0020$) (**Figure 3B**). Terminal myogenic differentiation ($p=0.0001$) was strongly decreased by 43.6 % in the DM groups not in mDM-derived MPCs (**Figure 3C**). Finally fusion was reduced in both sDM and mDM cells with a stronger impact on sDM MPCs (57.2 % and 28.9 %) ($p<0.0001$ for each) (**Figure 3B**). As a result, formation of myotubes was critically impaired in DM, as shown in **Figure 3C**. Since the purity of cells was variable from one patient to another, we checked that no correlation was found between purity of the samples (% of CD56⁺ cells) and the proliferation or fusion index in all MPC studied (**Figure 2D**), On the contrary, proliferation capacity of the cells was significantly correlated with their differentiation ($p=0.008$) and fusion ($p<0.0001$) capacities, which strongly suggests that impaired proliferation is an important defect in DM MPCs (**Figure 3D**).

While differentiation and fusion were less affected in mDM than in sDM, proliferation defects were similar in both subgroups. In addition, similar alteration in MPC proliferation was also observed in JDM-derived MPCs (**Figure 3B**, right bottom diagram). Altogether, these results support a general proliferation defects in DM MPCs that impacts myogenesis and occurs in a cell-autonomous manner.

Cellular aspects of MPC proliferation impairment in DM patients

Microscopy examination of MPC cultures under proliferation conditions showed an increased size of DM-derived MPCs as compared with HC-derived MPCs, together with enlargement of the nucleus (**Figure 4A**). Such alterations might indicate cell cycle alteration, return into quiescence and/or senescence. We investigated cell cycle status of MPCs derived from HC, sDM, mDM, and jDM using Pyronin Y/Hoechst staining and flow cytometry analysis in order to distinguish cells in G₀, G₁/S or G₂/M phases of the cell cycle (Abou-Khalil et al., 2013). No significant changes in the distribution of MPCs into the cell cycle were observed (**Figure 4B**). Since enlarged cell shape is also a feature of senescent cells, the proportion of MPCs that enter into senescence was evaluated using a fluorescent assay of beta-galactosidase activity and flow cytometry analysis. First, analysis of the cell size showed that sDM and jDM populations exhibited cell enlargement with an increase of the percentage of large cells (9.21 and 16.9% respectively) (**Figure 4C1**). Whatever the pathology, we observed a strong increase of beta-galactosidase activity in large cells, as compared to control samples (**Figure 4C4**), while small cells were not affected (**Figure 4C3**). In most severe form of DM, this impacted on the beta-galactosidase of the whole population (**Figure 4C2**). These results show an increase of senescence in DM-derived MPCs, which may explain the proliferation defect of the cells.

IFN involvement in DM-derived MPC proliferation impairment

Type I interferon (IFN-I) induction is a prominent feature in DM pathologies, and our previous studies have unveiled IFN-I signature in muscle-derived MPCs from JDM patients (Gitiaux, 2017). Moreover, it was recently shown that inhibition of IFN-I signaling could improve muscle function in DM patients refractory to conventional immunomodulatory treatments (Ladislau 2018). Therefore, we hypothesized that IFN-I pathway activation could be deleterious for MPC proliferation in adult DM.

We first investigated the IFN signature of DM-derived MPCs. Digital quantification of mRNAs of ISGs was performed in lysates from HC-, sDM- and aDM-derived-MPCs using a 6-genes signature: IFI27, IFI44L, IFIT1, ISG15, RSAD2, and SIGLEC1 (Pescarmona 2019). Global IFN score was not significantly increased in DM-derived MPC lysates. However, IFI27 and IFI44L transcript expressions were significantly increased in sDM-derived MPCs in

comparison with HC-derived MPCs, whereas slight but not significant increase in ISG15 expression was observed in sDM samples and other transcripts were not affected. Moreover, no increase of ISG expression was observed in mDM-derived MPCs (**Figure 5**).

Effects of IFN-I on MPC proliferation was assessed *in vitro* by performing recombinant human IFN- β dose-response experiments on HC-derived MPCs. Results show a dose-dependent inhibition of proliferation from 100 U/ml (-2 and -7.4% for 100 U/ml and 1000 U/ml; $p=0.0481$ and 0.0176 respectively), yet lower IFN concentration (10 U/ml) had a slight stimulatory effect (+4.4%) ($p=0.0485$) (**Figure 6A**). This result suggested possible inhibitory effect of IFN-I on DM-derived MPCs. We therefore tested whether inhibition of IFN-signaling would affect proliferation of DM-derived MPCs by using either an inhibitor of the JAK/STAT pathway (ruxolitinib) or a blocking antibody against the IFN-I receptor (IFNAR). Inhibition of IFN-I signaling pathway by ruxolitinib had significant inhibitory effects on HC derived-MPC proliferation ($p=0.0097$) (**Figure 6B**). On the contrary, both inhibitors significantly increased proliferation of sDM-derived MPCs by 14,5 and 16,75% ($p=0.001$ and $p=0.0026$, respectively) (**Figure 6C**), resulting in proliferation index close to that of untreated HC-derived MPCs (**Figure 6B**).

Discussion

This study identified an *in vitro* impairment of mDM, sDM and jDM derived-MPCs, with an alteration of the myogenesis process, particularly a strong impairment of their proliferative capacity. IFN-I involvement was supported by the up-regulation of ISG expression in MPC, and by the rescue of proliferation by IFN-I pathway blocking strategies.

Myogenesis process impairment

Myogenesis implemented by MPCs is tightly regulated by many factors, dependent on various intra or extracellular cues, the latter being mainly provided by the cell environment. DM and JDM are both idiopathic inflammatory myopathies, with an adult- or a pediatric-onset, respectively. In this work, the evidence of the maintenance of MPC dysfunction *in vitro*, remote from any inflammatory or cytokinic environment, supports the hypothesis of an intrinsic cell dysfunction. These pathological changes have not yet been described and could participate to the defect of regeneration of myofibers in DM muscles after the onset of the disease.

Myogenic properties of IIM-derived cells remain poorly described. Across the literature, only two works reported IIM derived-MPC analysis (Cseri et al., 2015; Hayashi et al., 1990). Starting from cell culture of a single DM patient muscle, Hayashi et al evaluated the myogenesis process. However, after dissection and enzymatic digestion, muscle was cultured without specific MPC sorting, resulting, as showed in the figures of the article, in a mix of various cell types and myotubes. The authors found no differences between control and DM muscle culture. The lack of MPC purification induces potential severe bias in MPC evaluation, with at least entanglement with other cell type functions that may distort the results of these studies. In the second work, primary MPCs derived from untreated (n=6) or corticosteroids treated IIM patients (n=3) and healthy control (n = 7) were compared. The authors identified an impaired proliferation and slightly altered fusion capacity in cultures from untreated IIM specimens. Cell proliferation was characterized by the increase in the number of myogenic nuclei normalized to the value at the 2nd day of culturing (Cseri et al., 2015). Nevertheless, this study supports our findings with myogenesis impairment of DM derived-MPCs.

Muscle stem cell pool in DM and HC muscle biopsies

In two published works, muscle stem cells pool, identified by PAX7 nuclei expression has been evaluated in jDM or IBM, DM and PM PAX7 nuclei cell in muscle biopsies (Baumann et al., 2018; Wanschitz et al., 2013). Number of satellite cells was significantly increased in inflammatory myopathies in comparison to control, and was correlated with the intensity of inflammatory infiltrates (Wanschitz et al., 2013). In the jDM study, PAX7 increase was associated with dysregulation of the myogenic regenerative pathway (i.e. MRF expression) in advanced muscle lesions (Baumann et al., 2018). These results are consistent with our results. In addition, we found that the increased number of PAX7 cells was prominent in the perifascicular area (i.e. the two first rows of cells on fascicule edge).

Involvement of IFN-I in DM and JDM pathogenesis

Occurrence of myositis, and particularly DM under IFN- α therapy, and recovery at treatment suspension/disruption led to the consideration of IFN involvement in IIM pathogenesis (Matsuya 1994) (Shiba et al., 2014). Involvement of IFN-I in DM and jDM pathogenesis was confirmed by histological studies (Illa et al., 1997; Salajegheh et al., 2010; Uruha et al., 2018) and transcriptomic analyses (Greenberg et al., 2002; Lundberg et al., 1995; Rice et al., 2017), and was finally correlated with myositis activity (Greenberg et al., 2012). Our work showed that adding IFN- β on normal cell culture mimicked proliferation impairment as observed in DM, while blockade of IFN-I downstream pathway rescued impaired proliferation of DM-MPCs, demonstrating that IFN is a major player in altered myogenesis in DM. Paradoxically, despite a significant upregulation of 2 over 6 ISGs in sDM-derived MPC lysates, IFN-I signature was not different between HC and DM derived-MPCs. This negativity can be explained by the variation of ISG expression regarding the tissue tested (i.e. ISG panel is different if evaluated on muscle biopsy, PBMCs or MPCs) and advocates for the need of tissue-specific IFN-I signature built through robust transcriptomic analysis (Gallay et al., 2019).

IFN and myogenesis process

The deleterious role of IFN- β on cell growth is well described. Indeed, IFN is known to decrease proliferation of tumor and virus-infected cells but also of normal cells (fibroblasts, lymphocytes and epithelial cells) by blocking the transition from G0/G1 into the phase S of the cell cycle (Hertzog et al., 1994). In this context, IFN- β is a better inhibitor than IFN- α (Hertzog et al., 1994) and among IFN-I, it is the most expressed in the blood of DM patients. After exposition to IFN-I, C2C12 mouse myoblasts and immortalized human MPCs show alteration in differentiation, myotubes having a lower surface than untreated myotubes (Franzi et al., 2013). After ISG15 silencing by siRNA, there is no more effect of IFN- β exposure on myotubes (Franzi et al., 2013). The muscle atrophy caused after IFN-I exposure or after poly(I:C) exposure (which activates the IFN pathway through TLR3 activation) on muscle cells is also found to be abolished by blocking the IFNAR receptor (Ladislau et al., 2018). To assess impact of IFN-I on normal MPC proliferation, we cultured HC derived-MPCs in the presence of IFN- β . Interestingly, the lowest concentration increased proliferation, meanwhile the 100 and 1000 U/ml concentrations decreased cell proliferation. These findings supported the fact that IFN-I and myogenesis interactions probably follow a nuanced range of variations, from beneficial to deleterious.

Despite the impairment of proliferation, our results found no particular blockade in cell cycle, arguing for a global decrease of proliferation. Regarding senescence, our results identified a quantitative and qualitative increase of in large cells, that expressed senescence markers. IFN- β has been demonstrated able to induce senescence in normal human diploid fibroblasts (IMR90) (Moiseeva et al., 2006), and could take part in the senescence induction of DM derived-MPCs.

Inhibition of IFN-I pathway

It has been shown that ruxolitinib increases myoblast differentiation and fusion, and prevents IFN- β -induced myotube impairment (Ladislau et al., 2018). We blocked JAK-STAT pathway by a specific inhibitor: ruxolitinib. Ruxolitinib equally inhibits JAK1, JAK2 and JAK3 (Zhou et al., 2014). Indeed, several pathways are involved in IFN-I signaling: JAK-STAT, CRKL, PI3K and NF κ B, MAPK (Hervas-Stubbs et al., 2011). The choice of JAK1-STAT1-STAT3 pathway has been made considering a previous study of myoblast proliferation (Sun et al.,

2007). Our results show that treating DM- and jDM derived-MPCs with ruxolitinib, as well as blocking the IFN-I receptor, improved their proliferation. These in vitro results support case reports describing refractory DM- and jDM-patients treated with JAK-STAT inhibitors, either ruxolitinib or tofacitinib, who presented clinical positive outcomes (Hornung et al., 2014; Kahn et al., 2018; Ladislau et al., 2018). Taken together, these results advocate for IFN-I induced DM or jDM derived-MPCs impairment, which can be partly corrected by IFN-I pathway blockade.

DM subgroups and pathogenesis

Despite the clear differences in muscle impairment between sDM and mDM, our results support the hypothesis of a common alteration of MPC proliferation in both diseases in comparison to HC-derived MPCs. These findings rise the question of systemic and soluble factor that may be increased regardless to the muscular impairment or severity of histologic damages, and may leave a print on the muscle stem cells. Adult and juvenile DM have known common pathogenesis process, but also differ on certain points (Baechler et al., 2011; Preuße et al., 2016). Our results sustain a shared alteration of cellular functioning of MPCs in both jDM and DM.

Conclusion

This work identified impairment of DM derived-MPCs, with a potential involvement of IFN-I underlying this process. These findings may explain at least partially that DM muscle impairment evolve chronically, regardless to the auto-antibody status.

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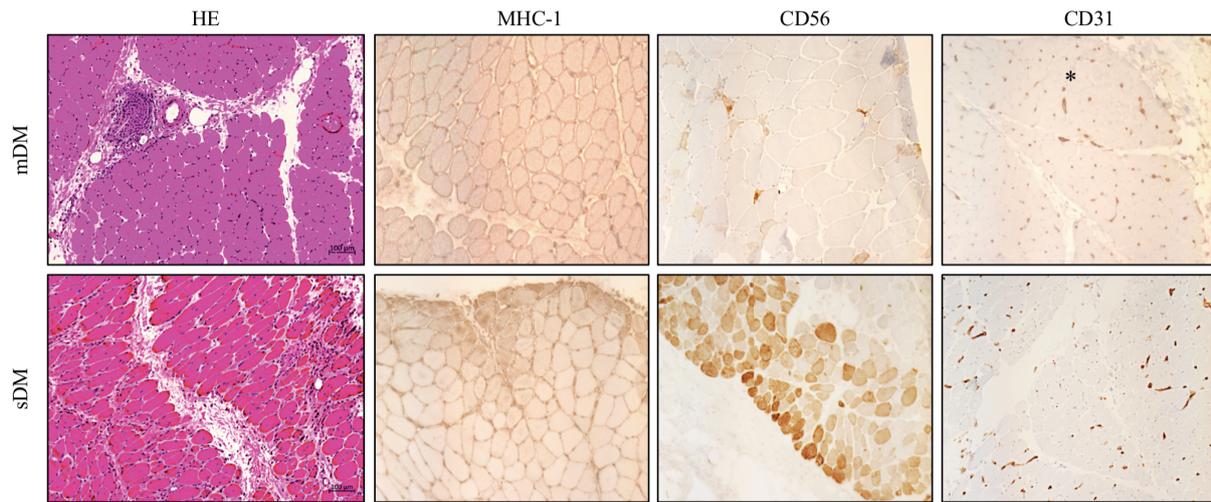


Figure 1. Histological assesment of sDM (severe dermatomyositis) and mDM (mild dermatomyositis). mDM (upper row) showed relatively preserved muscle in which perivascular inflammatory infiltrates and slight perifascicular atrophy on HE staining were observed, as well as diffuse Major Histocompatibility complex-1 sarcolemmal expression, sparse myofibers undergoing regeneration (CD56 positive myofibers), and focal capillary drop out (star in CD31 staining). sDM (lower row) showed important myofiber damage (heterogeneity in myofiber size, severe perifascicular atrophy, necrosis, myophagocytosis), inflammatory infiltrates, fibrosis, diffuse Major Histocompatibility complex-1 sarcolemmal expression with perifascicular enhancement, numerous myofibers undergoing regeneration with perifascicular enhancement (CD56 positive myofibers), and multiple capillary drop outs with related atrophy (CD31 staining).

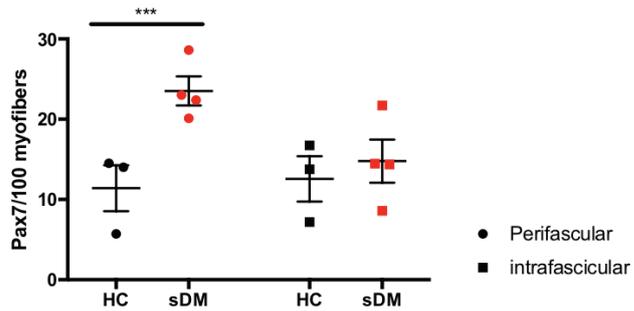
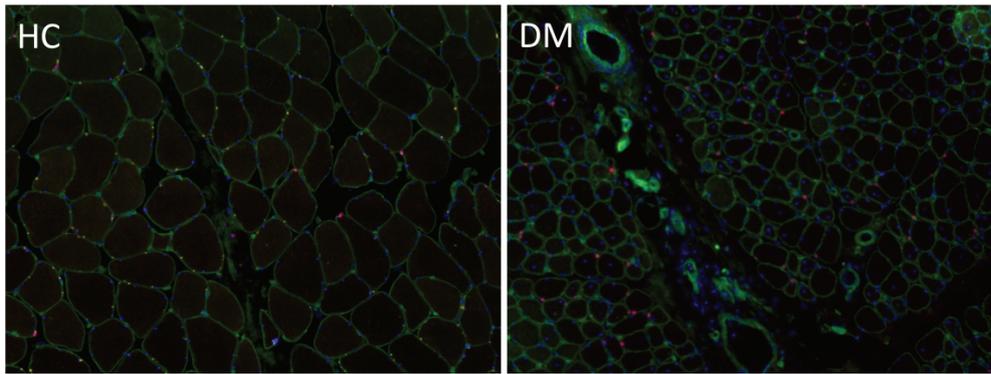


Figure 2. Pax7-positive cells in muscle sections of HC and DM muscle biopsies (green is laminin, red is Pax7 and blue is nuclei). Counting was distinguished between perifascular (2-3 first perifascicular myofibers) and the intrafascicular (rest of the fascicule) areas.

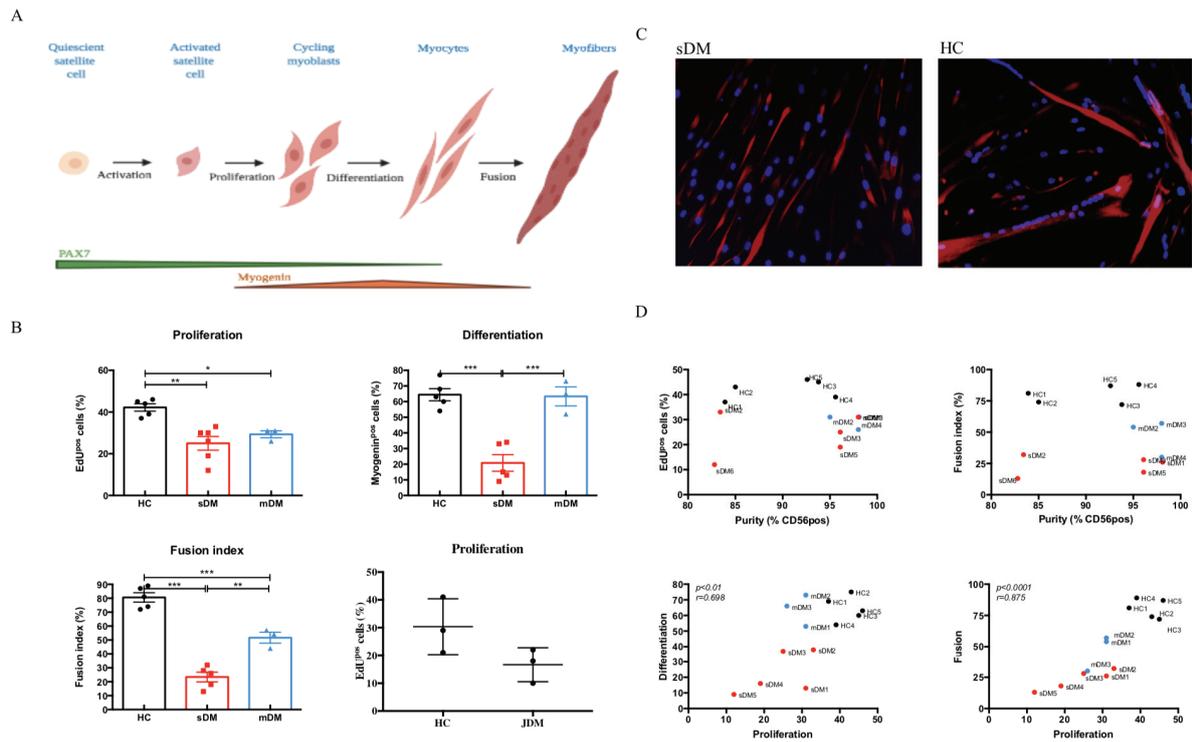


Figure 3. Myogenesis impairment in DM-derived MPCs. 2A. Myogenesis process. 2B. Proliferation (evaluated by percentage of EDU positive cells), differentiation (percentage of myogenin positive cells), and fusion (by index of fusion) in HC, DM, ADM and JDM derived-MPCs. 2C. Representative pictures of global myogenesis evaluation in HC and DM. Blue: nuclei, red: desmin. 2D. Correlation between CD56 purity and proliferation or fusion index and between proliferation and differentiation or fusion.

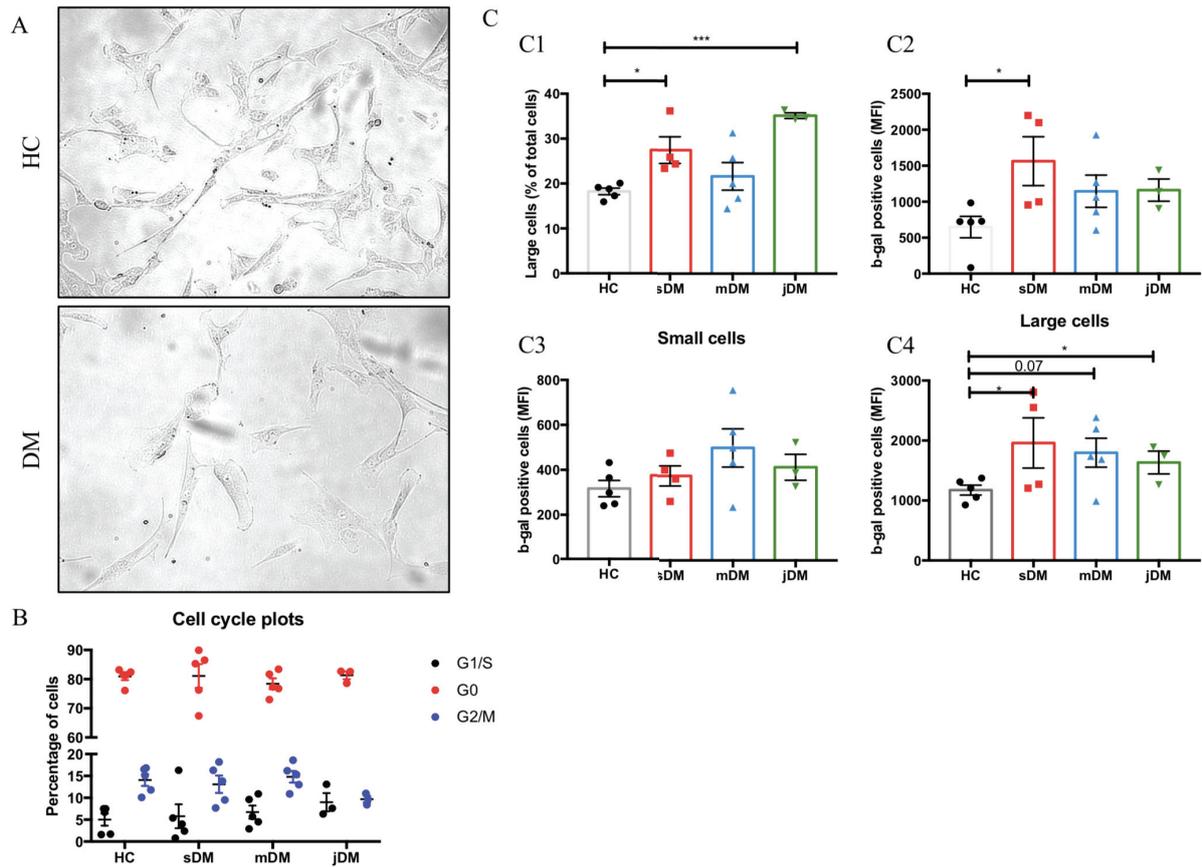


Figure 4. MPC senescence. 4A. Phase contrast of MPCs after 48 hours of proliferation in complete medium. 4B. Cell cycle analysis was performed by flow cytometry, and percentage of cells in G0, G1, G2 phases. 4C. Analysis of senescent cells by evaluation of cellular beta-gal expression using flow cytometry. C1: Percentage of large cells in the total MPC populations. C2,3,4: Percentage of beta-gal positive cells in total cell population, small and large cells, respectively.

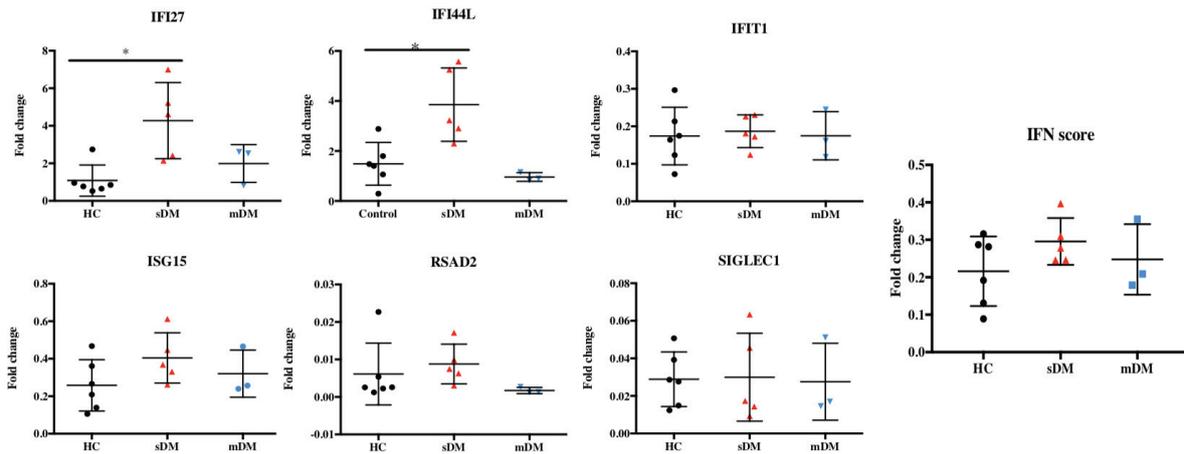


Figure 5. MPC interferon signature. MPC lysat was evaluated for type I IFN signature using Nanostring technology. The IFN signature is composed of 6 IFN stimulated genes (ISGs): SIGLEC1 (sialic acid binding Ig like lectin 1), IFI27 (interferon alpha inducible protein 27), IFI44L (Interferon induced protein 44 like), IFIT1 (interferon induced protein with tetratricopeptide repeats 1), ISG15 (interferon-stimulated gene 15) and RSAD2 (radical S-adenosyl methionine domain containing 2). Nanostring (NanoString Technologies) procedure hybridized 200 ng of RNA to the probes (a reporter probe and a capture probe) to evaluate level of transcripts of each ISG. Count number obtained for the 6 ISGs were normalized by the geometric mean of 3 housekeeping genes count numbers (β -Actin, HPRT1 (hypoxanthine phosphoribosyltransferase 1) and POLR2A (RNA polymerase II subunit A)) as well as the negative and positive controls values using nSolver software. The relative expression of each ISG was determined for each normalized ISG expression divided by the median normalized expression of each ISG from a control group. Finally, the median of these 6 ISG relative expression was used to calculate the IFN score. The threshold was previously defined at 2.3.(Pescarmona et al., 2019)

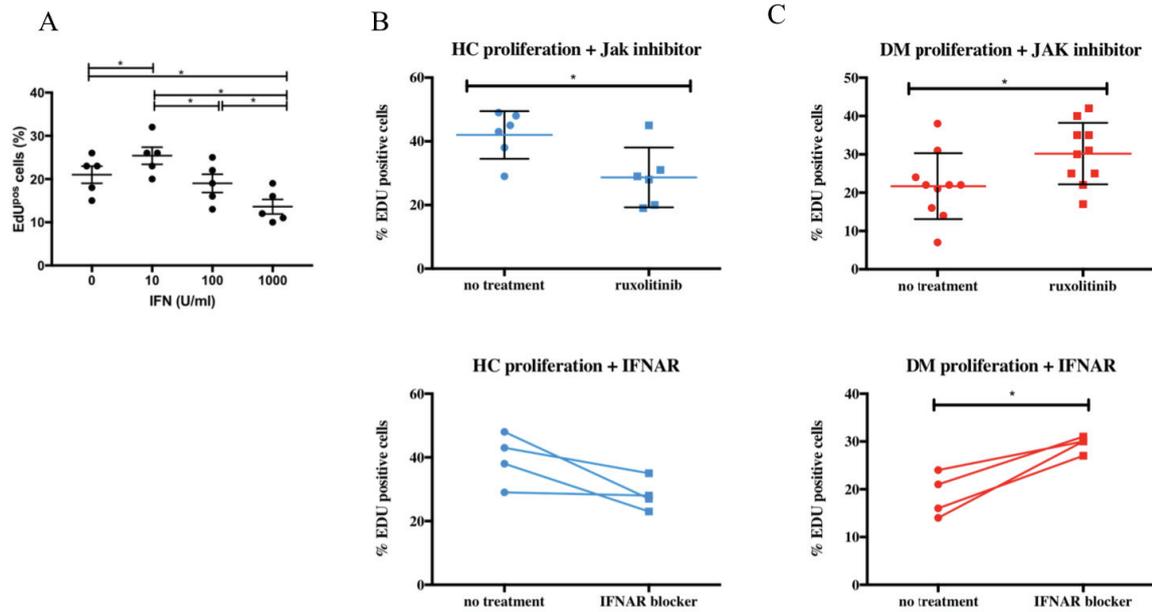


Figure 6. IFN-I and myogenesis. A: Effect of type I IFN on in vitro proliferation of HC-MPCs. IFN-I was added daily in the medium, and proliferation index was evaluated. B: Proliferation of HC derived-MPCs with and without JAK inhibitor (Ruxolitinib) or IFNAR blocker. C: Proliferation of DM (including sDM, mDM and jDM) derived-MPCs with and without JAK inhibitor (Ruxolitinib) or IFNAR blocker.

Patient	options	1	2	3	4	5	6	7	8
INFLAMMATORY DOMAIN									
endomysial inflammatory infiltrates	0, 1, 2	0	0	1	2	1	1	1	1
perimysial inflammatory infiltrates	0, 1, 2	0	0	0	2	2	2	0	1
perivascular inflammatory infiltrates	0, 1, 2	0	1	0	2	2	2	1	2
Inflammatory domain total	range from 0-6	0	1	1	6	5	5	2	4
VASCULAR DOMAIN									
Arterial abnormality	0 (N) or 1 (Y)	0	0	0	1	1	0	1	1
vascular domain total	range from 0-1	0	0	0	1	1	0	1	1
MUSCLE FIBRE DOMAIN									
Perifascicular atrophy	0 (N) or 1 (Y)	0	0	1	1	1	1	1	1
Fibre atrophy: non-perifascicular	0 (N) or 1 (Y)	0	0	1	1	1	1	1	1
Myofiber regeneration (IHC CD56)	0 (N) or 1 (Y)	0	1	1	1	1	1	1	1
Myofibers necrosis	0 (N) or 1 (Y)	0	0	0	1	1	1	1	1
sarcolemmal C5b9 deposits	0 (N) or 1 (Y)	0	1	1	1	0	0	1	1
Internal myonuclei in normal fibres	0 (N) or 1 (Y)	1	0	0	1	1	1	1	1
muscle fibre domain total	range from 0-6	1	2	4	6	5	5	6	6
CONNECTIVE TISSUE DOMAIN									
Any endomysial or perimysial fibrosis	0 (N) or 1 (Y)	0	0	0	1	1	1	1	1
Any endomysial or perimysial adiposis	0 (N) or 1 (Y)	0	0	0	0	0	1	0	1
Connective domain total	range from 0-2	0	0	0	1	1	2	1	2
VISUAL ANALOGIC SCALE - OVERALL IMPRESSION OF ABNORMALITY									
VAS score	range from 0-5	0.5	0.5	1	4	4	4	4	5
TOTAL DM SEVERITY SCORE									
Histologic DM severity score		1.5	3.5	6	18	16	16	14	18
CLASSIFICATION		mDM 2	mDM 3	mDM 4	sDM1	sDM2	sDM3	sDM5	sDM6

Table 1. Histologic DM severity score for the 8 patients included in the study. DM diagnosis was confirmed after histology analysis when at least 2 of the 3 following criteria were present: inflammatory infiltrates (identified on HE staining), capillary dropout (identified on CD31 staining) and MHC-I overexpression. The latter essential point do not appear in the histologic DM severity score.

DM severity score was set up using a four-domain analysis adapted from Varsani et al, 2015 (score instructions are available in Suppl tab. 1). Histologic DM severity score correspond to the addition of the 4 domains total quote. This severity score delineated 2 distinct subgroups: severe and mild DM (respectively sDM and mDM), with a severity score <8 for mDM and >12 for sDM. DM: dermatomyositis, IHC: immunohistochemistry, N: no, VAS: visual analogic scale, Y: yes

CLINICAL AND BIOLOGICAL DATA	mDM2	mDM3	mDM4	sDM1	sDM2	sDM3	sDM5	sDM6
Sex	M	M	F	M	F	F	M	F
Age	66	39	72	63	57	23	59	28
Muscle weakness (MRC testing, proximal/distal)	5 /5	5/ 5	4+/5 prox	3-/5 prox	4/5 prox	4-/5 prox	4/5 prox	4/5 prox
Maxmimal creatine kinase (UI/L)	400	300	1000	560	780	1200	6500	370
Myostis specific auto-antibody	MDA5	MDA5	Mi2	/	MDA5	Mi2	SAE2	MDA5

Table 2. DM patient demographical, clinical and serological characteristics. DM: dermatomyositis, F: Female, M: Male, mDM: mild DM, prox: promimal weakness, sDM: severe Dm

HISTOLOGIC DM SEVERITY SCORE	Options	Definitions and Instructions	Mandatory for DM diagnosis	severity scoring
INFLAMMATORY DOMAIN				
Presence of inflammatory infiltrates on the muscle biopsy	0 (N) or 1 (Y)	Presence of inflammatory infiltrates in endomysial, perimysial and/or perivascular area. Absence – N; Presence – Y	1	
endomysial inflammatory infiltrates	0, 1, 2	For each of endomysial, perimysial, perivascular distributions, score for inflammatory infiltrating cells as follows: if none, or less than 4 cells in x20 field - score 0; if >4 cells in a x20 field and/or 1 cluster (where a cluster is approx 10 cells or more) - score 1; if >2 clusters in whole biopsy, and/or diffusely infiltrating cells (ie >20 cells in a 20x field) - score 2		1
perimysial inflammatory infiltrates	0, 1, 2			1
perivascular inflammatory infiltrates	0, 1, 2			1
Inflammatory domain total	Possible range 0-6			
VASCULAR DOMAIN				
Capillary dropout (Using immunohistochemistry for CD31) or C5-b9 capillaries deposits	0 (N) or 1 (Y)	Obvious and marked decrease in the density of capillary network, AND/OR C5-B9 capillaries depositis, not restricted to areas of perivascular atrophy. Absence – N; Presence – Y	1	
Arterial abnormality	0 (N) or 1 (Y)	Mural thickening and/or endothelial swelling and/or transmural inflammation in arteries/arterioles. Absence – N; Presence – Y		1

vascular domain total	Possible range 0-1			
MUSCLE FIBRE DOMAIN				
MHC Class I over- expression	0 (N) or 1 (Y)	Presence of MHC class I staining on or in muscle fibres. Absence – N; Presence – Y	1	
Perifascicular atrophy	0 (N) or 1 (Y)	Affecting > 6 fibres out of 10 along one edge of a fasciculus, not exclusive to type IIb fibres. Absent - score N. Present - score Y.	1	
Fibre atrophy: non-perifascicular	0 (N) or 1 (Y)	Fibre atrophy: non-perifascicular (outside normal variation for age). Affecting > 6 fibres out of 10 in the center of a fasciculus, not exclusive to type IIb fibres. Absent - score N. Present - score Y.	1	
Myofiber regeneration (using immunohistochemistry for CD56)	0, 1, 2	Less than 6 positive fibres in a x20 field - score N; > 6 positive fibres in a x20 field - score Y	1	
sarcolemmal complement (C5b9) deposits	0 (N) or 1 (Y)	Sarcolemmal complement membrane attack complex (C5b9) deposits on non-necrotic fibers. Absent - score N. Present on one or more non-necrotic fiber - score Y.	1	
Myofibers necrosis	0 (N) or 1 (Y)	Less than 6 positive fibres in a x20 field - score N; > 6 positive fibres in a x20 field - score Y	1	
Internal myonuclei in non-basophilic otherwise normal fibres	0 (N) or 1 (Y)	Internal myonuclei in non-basophilic cells (in otherwise normal fibres) in 1 or more fasciculi excluding myotendinous junctions. If < 3% fibres - score 0. If > 3% fibres - score 1	1	
muscle fibre domain total	Possible range 0-6			
CONNECTIVE TISSUE DOMAIN				

Any endomysial or perimysial fibrosis	0 (N) or 1 (Y)	For fibrosis in each of endomysial and perimysial distributions, score as follows: Absence: score N. Presence of any: score Y	1
Any endomysial or perimysial adiposis	0 (N) or 1 (Y)	For adiposis in each of endomysial and perimysial distributions, score as follows: Absence: score N. Presence of any: score Y	1
Connective domain total	Possible range 0-2		
OVERALL HISTO-PATHOLOGISTS' VAS SCORE DATA			
Visual analogue scale - overall impression of abnormality	Possible range 0-5		1

Supplementary table 1. Set value and explanation for the filing of histologic DM severity score. Y: yes, N: no

1.3 Additional results

Additional investigations were made to the second part of PHD work in order to:

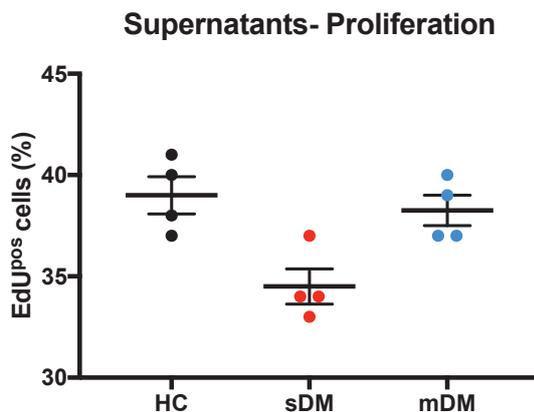
- determine if altered capacities of MPCs to proliferate, to differentiate and to fuse could be due to a soluble factor acting in an autocrine or paracrine way (Additional figure 1).
- evaluate if the intrinsic MPC alteration is specific to DM, or if this impairment may be common to other IIMs (Additional figure 2).
- further explore the cellular and molecular mechanisms underlying the intrinsic MPC alterations and strengthen the IFN-I involvement in DM pathogenesis (Additional figures 3, 4 and 5).

1.3.1 Analysis of HC derived-MPC proliferation in the presence of supernatant from DM derived-MPC cultures

In order to study the presence of soluble factors potentially secreted by MPCs themselves, we evaluated the proliferation of HC-derived MPCs incubated for 24 h with sDM, mDM or HC derived-MPC supernatants.

Supernatants were collected after 48h of culture of sDM, mDM or HC derived-MPCs seeded at 3000 cells/cm² in complete medium (Mix C-39365 + FBS 10%). Proliferation was then evaluated by the addition of the nucleotide analogue EdU for 6 hours,.

HC derived-MPCs had a significantly lower proliferation when incubated with sDM derived-MPC supernatant in comparison with HC and mDM derived-MPC supernatants (p=0.0228) (additional figure 1),



Additional figure 1. Proliferation index of HC derived-MPCs incubated with sDM, mDM or HC derived-MPC supernatants.

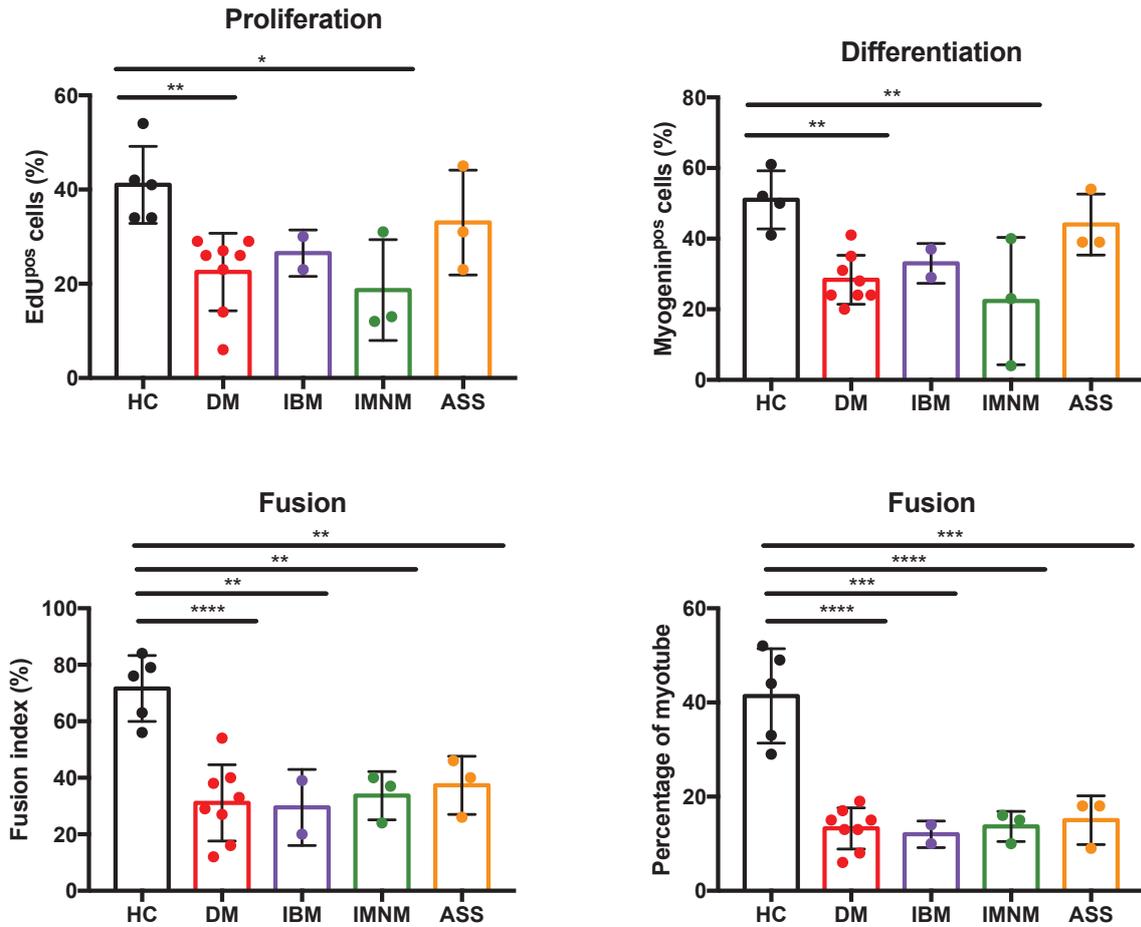
These results support the hypothesis that sDM derived-MPCs are responsible for an autocrine or paracrine production of soluble factors that altered proliferation.

1.3.2 Evaluation of myogenic properties of Inclusion body myositis, immuno-mediated necrotizing myopathy and anti-synthetase syndrome derived-MPCs.

Regarding the results obtained for DM-derived MPCs, and considering the similar chronic muscle weakness observed in patients with other IIM, we have extended the investigation to other IIM derived-MPCs: immune-mediated necrotizing myopathy (IMNM) (n=3), inclusion body myositis (IBM) (n=3), and anti-synthetase syndrome (ASS) (n=3).

MPC proliferation was assessed by the incorporation into DNA of a nucleotide analogue, EdU after 6 h of incubation in complete medium. We assessed differentiation after 3 days of MPC culture in differentiation medium by using immunofluorescent labeling of Myogenin, a transcription factor which is expressed only at the time of terminal myogenic differentiation. Cell fusion was quantified as fusion index and as percentage of myotubes.

Results show that DM- and IMNM-derived MPCs exhibited a significant lower proliferation index than HC-derived MPCs ($p=0.0007$ and 0.0108 , respectively) (additional figure 2). The difference was not significant for IBM- and ASS-derived MPCs in comparison to HC-derived MPCs. Differentiation index was significantly decreased in DM and IMNM as compared with HC ($p=0.0053$ and $p=0.0047$ respectively) (additional figure 2). The difference did not reach significance for IBM- and ASS-derived MPC differentiation in comparison to HC-derived MPCs. The fusion index and percentage of myotubes were significantly decreased ($p=0.0003$ and $p<0.0001$) for all studied IIM-derived MPCs (DM, IBM, IMNM and ASS).



Additional figure 2. Assessment of each step of in vitro myogenesis (including proliferation, differentiation and fusion) in HC, DM, IBM, IMNM and ASS derived-MPCs.

These results show that myogenesis is impaired for every IIM subtype derived MPCs. Interestingly, the impairment takes place at various steps depending on IIM subgroup. Indeed, DM- and IMNM-derived MPC myogenesis was impaired at an early step of the process (i.e., proliferation), whereas IBM and ASS derived-MPCs were not able to complete myogenesis process during the latest step (i.e. fusion).

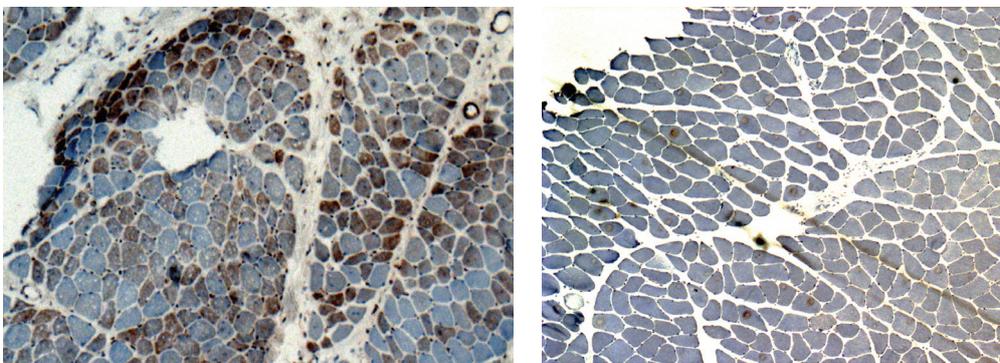
Further investigations focused on the cellular and molecular mechanisms underlying alterations of MPC myogenic properties (Additional figure 3, 4, 5 and 6) through histological analysis of MxA on patient and control muscle biopsies, evaluation of IFN- β deleterious effects on proliferation of HC-derived MPCs, measurement of ISG expression as well as several pro-inflammatory cytokines transcripts using Nanostring technology on HC-, DM-, IBM-, IMNM- or ASS-derived-MPC lysates.

1.3.3 Histological evaluation of MxA, an interferon stimulated gene, overexpression by sDM myofibers, an *ex vivo* study

MxA, an ISG, was defined as a valuable histological tool for DM diagnostic (Uruha et al., 2017), and its expression supports implication of IFN-I in DM pathogenesis. In my PhD work, DM-derived-MPC myogenesis impairment was rescued by IFN-I signaling inhibition with the JAK inhibitor ruxolitinib or IFN-I receptor blocking antibodies. Considering our findings, we performed evaluation of MxA staining in DM muscle biopsies of the severe cases to confirm the activity of IFN-signaling.

MxA immunostaining was performed for 4 sDM and 4 HC muscles, using a protocol kindly provided by Dr Léonard (Hôpital Pitié Salpêtrière, Paris, France) that uses monoclonal anti-MxA antibodies (Mx1/2/3 [H-285, sc-50509], 1:100 dilution, Rabbit, Poly IgG, Santa Cruz Biotechnology, Dallas, TX). MxA staining in the cytoplasm was considered positive except for necrotic fibers.

Positive MxA staining was identified for the 4 sDM patients, with a cytoplasm staining of numerous myofibers with diffuse overexpression, and perifascicular enhancement. No positive myofibers was found in HC muscle (additional figure 3).



Additional figure 3. MxA cytoplasmic overexpression analysis in sDM (left) and HC (right) muscle.

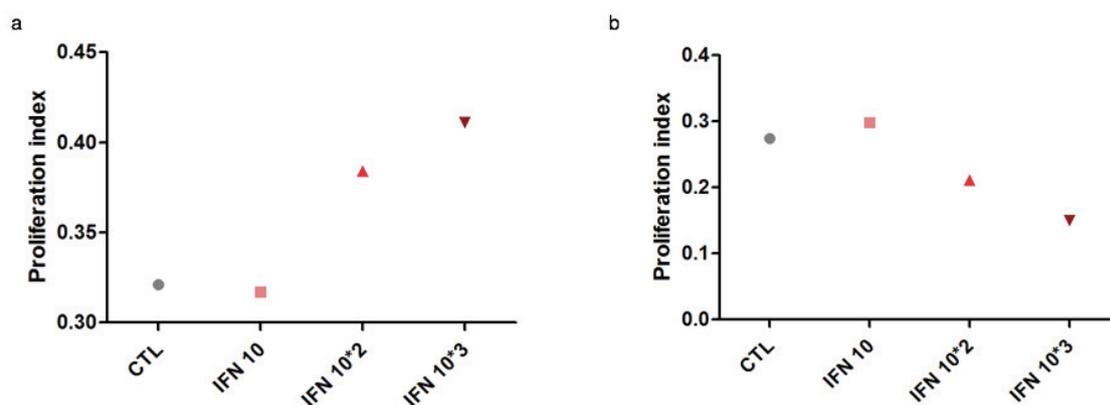
These findings confirm *in vivo* the involvement of IFN-I in muscle dysfunction of sDM.

1.3.4 Setup for the assessment of IFN- β deleterious effects on proliferation of HC-derived MPCs

In the hypothesis that myogenesis impairment in DM and jDM muscles is caused by activation of the IFN pathway, we tried to reproduce proliferation impairment *in vitro* by activating the IFN-I pathway.

HC-derived MPCs were treated with recombinant IFN- β in order to evaluate proliferation. Three different concentrations of IFN- β were used (10 U/ml, 10^2 U/ml, 10^3 U/ml), defined in accordance to previous studies (Ladislau et al., 2018; Franzi et al., 2011). IFN-I was added daily in complete medium (SMGM Promocell 23060 + P/S 1 % + Mix C-39365 + FBS 10% (Fetal Bovine Serum) or low FBS medium (SMGM Promocell 23060 + P/S 1 % + FBS 0.5%) to investigate potential interactions with the effectors contained in the serum.

In complete medium culture conditions HC-derived MPCs showed increased proliferation proportionally to the IFN- β doses (additional figure 4a), while proliferation was decreased in inverse proportion to IFN- β doses in low FBS condition (additional figure 4b).



Additional figure 4. Proliferation quantification of HC-derived MPCs treated by different IFN-I concentration range and set up in high (a) and low (b) FBS culture conditions.

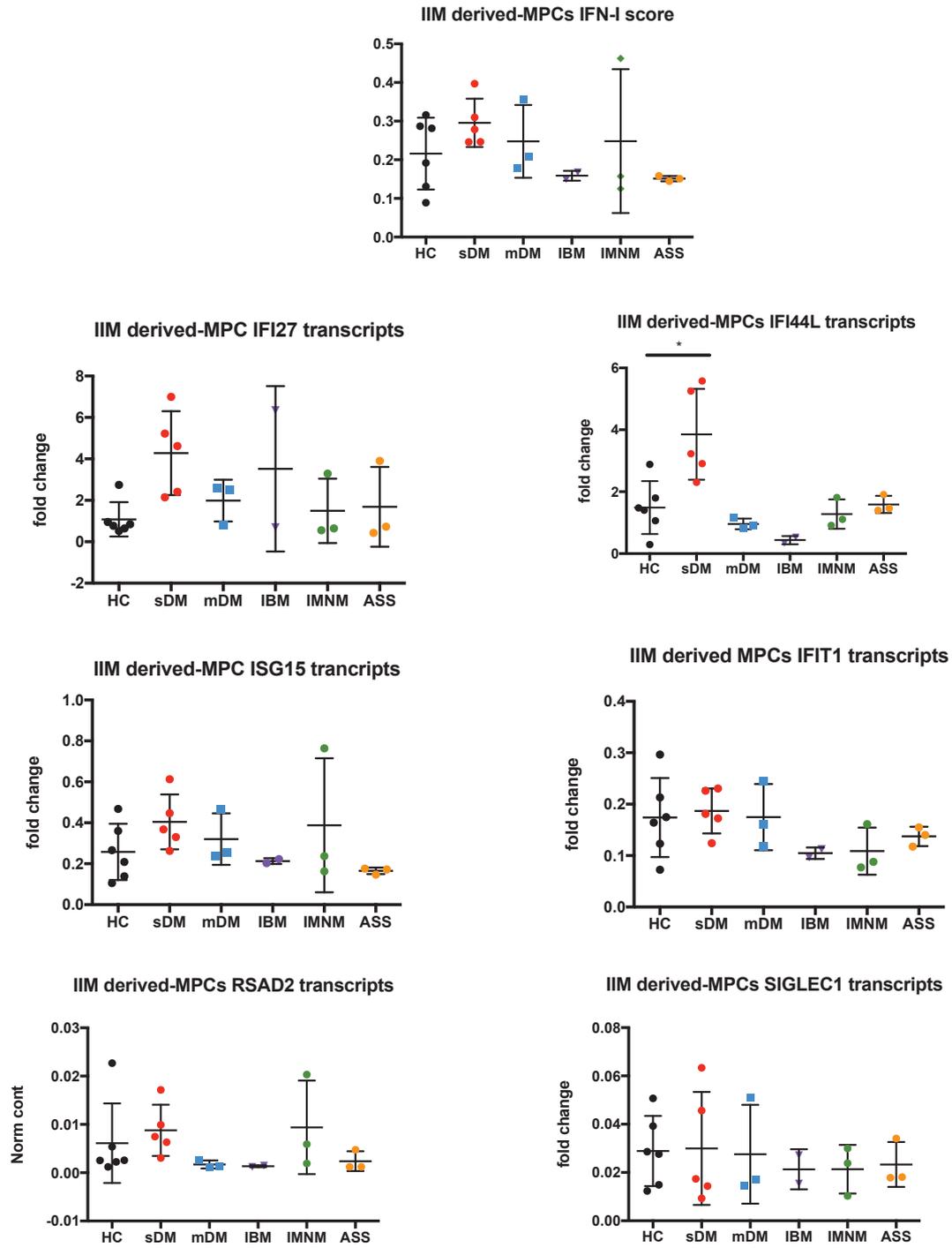
After contacting authors of the last publication reporting myogenesis impairment under IFN-I treatment (Ladislau et al., 2018), the experimental set up was clarified with low dose of FBS in the medium (only 0.5%) These results support that FBS, which contains huge quantity of various effectors, including potent mitogens, may have masked or may have interact with the effect of IFN-I.

1.3.5 Measurement of ISG transcripts using Nanostring technology on HC-, DM-, IBM-, IMNM- or ASS-derived-MPC lysates

As seen previously, MPCs derived from each IIM patient presented a global impairment of myogenesis. In order to further investigate the molecular pathway that underlie this myogenesis alteration, and considering the known implication of IFN-I in DM derived-MPC impairment, we performed evaluation of ISG expression in HC, DM, IBM, IMNM and ASS derived-MPC lysates.

The expression of 6 Interferon Sensitive Genes (ISGs) was evaluated according to Pescarmona et al, 2019: SIGLEC1 (sialic acid binding Ig like lectin 1), IFI27 (interferon alpha inducible protein 27), IFI44L (Interferon induced protein 44 like), IFIT1 (interferon induced protein with tetratricopeptide repeats 1), ISG15 (interferon-stimulated gene 15) and RSAD2 (radical S-adenosyl methionine domain containing 2). The 6 ISG expression was normalized by the geometric mean of 3 housekeeping genes count number (β -Actin, HPRT1 [hypoxanthine phosphoribosyltransferase 1] and POLR2A [RNA polymerase II subunit A]). The median of these 6 ISG relative expression was used to calculate the IFN score. The threshold was first defined as the mean of control group IFN score+2SD corresponding to a score of 2.3. The IFN score was evaluated in vitro in HC (n=6), sDM (n=5), mDM (n=3), IBM (n=2), IMNM (n=3) and ASS (n=3) derived-MPCs.

Global IFN score was not significantly upregulated in all IIM-derived MPC lysates. Data shown for sDM and mDM are those reported in our submitted manuscript. IFI27 and IFI44L transcript expression were significantly increased in DM-derived MPCs in comparison with HC-derived MPCs. ISG15 and RSAD2 transcripts were increased but do not reach the significance in sDM and IMNM derived-MPCs. IFIT1 and SIGLEC1 were not different (additional figure 5).

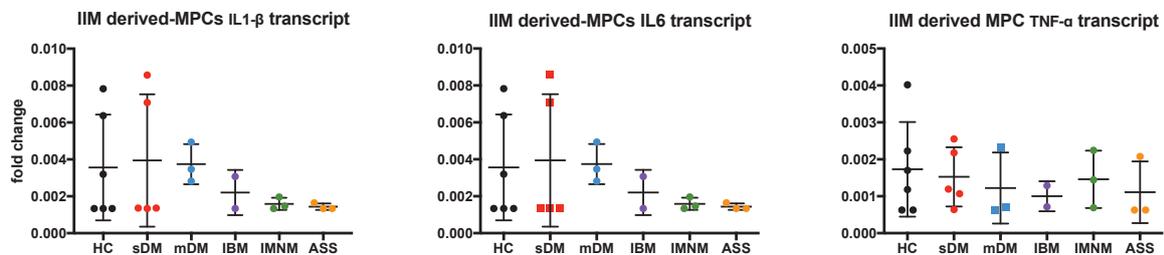


Additional figure 5. Transcriptional evaluation of ISGs with nanostring technology for HC, DM, IBM, IMNM and ASS derived-MPC lysates.

1.3.6 Evaluation of transcriptional expression of IL1- β , IL6 and TNF- α in HC, DM, IBM, IMNM and ASS derived-MPC lysates

IL1- β , IL6 and TNF- α are pro-inflammatory cytokines known to be involved in IIM pathogenesis (Archanapalli et al., 2015). In order to evaluate the involvement of such cytokines in the myogenesis impairment of IIM-derived MPCs, IL1- β , IL6 and TNF- α expression analysis was performed as part of the Nanostring experimentation.

No consistent increase or decrease of IL1- β , IL6 and TNF- α transcripts was found in IIM-derived MPCs, setting aside this molecular hypothesis (additional figure 6).



Additional figure 6. IL1- β , IL6, TNF- α transcripts analysis in IIM derived-MPC lysates.

DISCUSSION

1.1 Focal myositis: new insights in diagnosis and pathology: discussion.

As discussed within the publication, despite the small cohort presented, which reflected the rarity of the disease, our work allows to identify important points regarding this clinical entity. Associated disorders were noticeable, notably malignancy and immune mediated inflammatory diseases. Amongst the latter, Behcet's disease was surprisingly found in 5 cases of our cohort (14%) and should to be kept in mind for the clinical practice. Our work also highlights the potential recurrent outcomes, and argues for the need of FM patients follow up.

1.2. Impairment of adult myogenesis by muscle stem cells in IIM: discussion

1.2.1. DM pathological severity gradation scoring

Pathological scoring of DM cases allowed us to identify variations in the histological muscle damages when cases fulfilled several basic pathological criteria (i.e., inflammatory infiltrates, capillary dropout (identified on CD31 staining) and MHC-I sarcolemmal overexpression). Inspired by jDM consensual pathological scoring (Varsana et al., 2017), we have created an objective tool, focused on pathological severity gradation of the disease, which could be further used for other clinical or therapeutic DM studies. Considering the present study, apart from one patient, the main clinico-biological data were consistent with our defined scoring.

1.2.2. Muscle stem cells pool in DM and HC muscle biopsies

In two published works, muscle stem cell pool, identified by PAX7 nuclei expression, has been evaluated in jDM or IBM, DM and PM muscle biopsies (Baumann et al., 2018; Wanschitz et al., 2013). The number of satellite cells was significantly increased in inflammatory myopathies in comparison to control, which was correlated with the intensity of inflammatory infiltrates (Wanschitz et al., 2013). In the jDM study, PAX7 increase was associated with dysregulation of the myogenic regenerative pathway (i.e., MRF expression) in advanced muscle lesions (Baumann et al., 2018). Our data are consistent with these results. In addition, we found that the increased number of PAX7 cells was prominent in the perifascicular area (i.e., the first two rows of cells on fascicule edge).

1.2.3. Myogenesis process impairment

Myogenesis is tightly regulated by many factors, dependent on various intra or extracellular cues, the latter being provided by the cell environment. DM and jDM are both idiopathic inflammatory myopathies, with an adult- or a pediatric-onset, respectively. In studying myogenesis of DM- or jDM-derived MPCs, we showed that proliferation, differentiation and fusion were impaired. In this work, the maintenance of MPC dysfunction *in vitro*, remote from any inflammatory and cytokinic environment, supports the hypothesis of an intrinsic cell dysfunction. These pathological changes have not been described yet and could participate to the defect of regeneration of myofibers after the onset of the disease.

Regarding our findings of global myogenesis impairment in DM, we extended the study to other IIMs (IBM, IMNM and ASS). For all IIM-derived MPCs, myogenesis was globally impaired. Nevertheless, the results regarding proliferation and commitment into differentiation were heterogeneous, with a proliferation and differentiation significantly impaired in IMNM, and slightly decreased for ASS derived-MPCs. Insignificant myogenesis alterations in IBM could be explained by the small sample size, with only 2 patients. Two other IBM samples were tested but CD56 sorting was not possible, thus making their evaluation impossible.

Occurrence of regenerative basophilic fibers containing internal nuclei as a signal of myogenesis ongoing process is widely described in IIM muscle biopsy, and foremost in DM (Dalakas, 2015). Additional characterization of regenerative myofibers are usually reported by CD56 staining or neonatal myosin overexpression (Gitiaux et al., 2016). Myogenic properties of such cells remain poorly described. Across the literature, only two works reported IIM-derived MPC analysis (Hayashi et al., 1990; Cseri et al., 2015). Starting from cell culture of a single DM patient muscle, Hayashi et al evaluated the myogenesis process. However, after dissection and enzymatic digestion, muscle cells were cultured without specific MPC sorting, resulting, as shown in the figures of the article, in a mix of various cell types and myotubes. The authors found no differences in culture between control and DM muscles. In the second work, primary MPCs derived from untreated IIM patients (n = 6), corticosteroid treated IIM patients (n = 3) and healthy controls (n = 7) were compared. The authors identified an impaired proliferation and slightly altered fusion capacity in the primary cell cultures from untreated IIM specimen (Cseri et al., 2015). Off note, the lack of MPC purification induced potential severe bias in myoblast evaluation, with at least entanglement with other cell type functions that may distort the results of these studies. Finally, our work is the first study that evaluated properly and accurately the myogenic properties of IIM-derived MPCs *ex vivo*.

1.2.4. Impaired myogenesis process, as part of the downward spiral of the IIM pathogenesis?

The involvement of regenerating muscle cells in IIM pathogenesis has been proposed by Casciola-Rosen et al (Casciola-Rosen et al., 2005). In this work, analysis of IIM muscle biopsies identified that regenerating myocytes (identified by NCAM and neonatal myosin heavy chain) were overexpressing myositis specific autoantigens (catalytic subunit of DNA-dependent protein kinase, histidyl tRNA synthetase). Such autoantigen overexpression was

recapitulated in undifferentiated MPCs *in vitro*. This was reproduced in another study of muscle regeneration in mice acutely injured (Sciorati et al., 2014). Through these observations, authors pointed the fact that regeneration process may take part in the chronicization of autoimmune myositis. Indeed, increased autoantigen expression in myogenic cells accomplishing repair of firstly damaged myofibers could lead to an amplification cycle by long-lasting presence of the “triggers” of adapted and innate immune responses (Suber et al., 2008).

1.2.5. Soluble factors that impairs myogenesis

The analysis of the impact of sDM-derived MPC supernatant on HC-derived MPCs drove the consideration of a soluble factor involved in MPC myogenesis impairment. Interestingly, MPCs derived from sDM were producing more deleterious supernatant than mDM-derived MPCs. This questions the fact that surrounding damaged myofibers may secrete deleterious soluble factors that may prime nearby muscle stem cells, then resulting in intrinsically altered MPCs. One hypothesis is that the soluble factor could be IFN-I, as it was demonstrated that HC-derived MPCs were able to secrete IFN- β themselves after stimulation (Tournadre et al., 2012); and that jDM derived-MPCs secrete IFN- α (Gitiaux et al., 2018).

1.2.6. Involvement of IFN-I in DM and jDM pathogenesis

Occurrence of myositis, and particularly DM, under IFN- α therapy and recovery after treatment suspension/disruption led to the consideration of IFN involvement in IIM pathogenesis (Shiba et al., 2014). Such involvement of IFN-I in DM and jDM has been confirmed by histological studies (Illa et al., 1997; Salajegheh et al., 2010; Uruha et al., 2018) and transcriptomic analyses (Greenberg et al., 2002; Lundberg et al., 1995; Rice et al., 2017), and was finally correlated with myositis activity (Greenberg et al., 2012). Our work showed that adding IFN- β on normal cell culture mimicked proliferation impairment as observed in DM, while blockade of IFN-I downstream pathway rescued impaired proliferation of DM-derived MPCs, demonstrating that IFN is a major player in altered myogenesis in DM. Paradoxically, despite a significant upregulation of 2 over 6 ISGs in sDM-derived MPC lysates, IFN-I signature was not different between HC and DM derived-MPCs. This can be explained by the variation of ISG expression regarding the tissue tested (i.e. ISG panel is different if evaluated on muscle biopsy, PBMCs or

MPCs) and advocates for the need of tissue-specific IFN-I signature built through robust transcriptomic analysis (Gallay 2019).

1.2.7. IFN and the myogenesis process

The deleterious role of IFN- β on cell growth is well described. Indeed, IFN is known to decrease proliferation of tumor and virus-infected cells but also that of normal cells (fibroblasts, lymphocytes and epithelial cells) by blocking the transition from G0/G1 into the phase S of the cell cycle (Hertzog et al., 1994). IFN- β is a better inhibitor than IFN- α (Hertzog et al., 1994) and among IFN-I, it is the most expressed in the blood of DM patients. After exposition to IFN-I, C2C12 mouse myoblasts and immortalized human MPCs show alteration in differentiation, myotubes having a lower area than untreated myotubes. (Franzi et al., 2013) After ISG15 silencing by siRNA, there is no more effect of IFN- β exposure on myotubes (Franzi et al., 2013). Muscle atrophy caused after IFN-I exposure or after poly(I:C) exposure (which activates the IFN pathway through TLR3 activation) on muscle cells was described by Ladislau and colleagues. This effect was abolished by blocking the IFNAR receptor (Ladislau et al., 2018). Tournadre et al (2012) demonstrated in vitro, that during myogenesis process, MPCs activated by poly(I-C), the ligand of TLR-3, overexpressed MHC-1. This recapitulates the overexpression seen in DM histology, and interestingly, this overexpression was decreased after IFN- β blockade.

To assess the impact of IFN-I on normal MPC proliferation, we cultured HC-derived MPCs in the presence of IFN- β . We chose three different concentrations of IFN- β based on previous studies (Ladislau et al., 2018; Franzi et al., 2013). Interestingly, the lowest concentration increased proliferation, meanwhile the 100 and 1000 U/ml concentrations decreased cell proliferation. These findings supported the fact that IFN-I and myogenesis interactions probably follow a nuanced range of variations, from beneficial to deleterious.

1.2.8. Inhibition of IFN-I signaling

Following the hypothesis of a negative feedback loop implicating IFN-I, we blocked IFN- α receptor (IFNAR) in DM-derived MPC cultures and found positive effects on MPC proliferation. For clinical reasons, we next evaluated IFN-I signaling blockade. Several

pathways are involved in IFN-I signaling: JAK-STAT, CRKL, PI3K and NFκB, MAPK (Hervas-Stubbs et al). We chose to block JAK-STAT pathway considering previous study on myoblast proliferation that demonstrated the JAK1-STAT1-STAT3 pathway involvement in myoblast proliferation and differentiation regulation (Sun et al., 2007). For that we used ruxolitinib that equally inhibits JAK1, JAK2 and JAK3 (Zhou et al., 2014). Ruxolitinib was previously shown to prevent IFN-β-induced myotube impairment by increasing myoblast differentiation and fusion (Ladislau et al., 2018). Moreover, such inhibitors are available for patients. Our results showed that treating DM- and jDM-derived MPCs with ruxolitinib improved their proliferation and their differentiation. These in vitro results support clinical case reports of positive outcome in refractory DM- and jDM-patients treated with JAK-STAT inhibitors, either ruxolitinib or tofacitinib (Ladislau 2018; Kahn et al., 2018; Hornung et al., 2014). Taken together, these results advocate for IFN-I induced DM- or jDM-derived MPCs impairment, partly corrected by IFN-I pathway blockade.

CONCLUSION & PERSPECTIVES

Regarding the clinical research, the findings of the present study lead us to consider FM as a clearly identifiable entity, even if the question of whether FM is a syndrome or a disease per se remains to be answered. We describe new clinical and pathologic features to further define FM, enabling to identify different subsets. Identification of this entity, and its associated disorders, appears important for patient management, as it may be a harbinger of malignancy. Further investigations of interest could be to evaluate the characteristics of FM when associated with other disorders, notably cancer or Behcet's Disease.

Then, considering the translational research focused on muscle regeneration in IIM. Our study identified an intrinsic impairment of DM (mild, severe and juvenile)-derived MPCs, with a global alteration of the myogenesis process, with a particularly focus on the first step of this process that is proliferation. IFN-I involvement in the DM derived-MPC impairment was supported by: i) ISG up-regulation in IIM-MPC lysates, ii) the possibility to reproduce *in vitro* myogenesis impairment by incubating HC-derived MPCs with IFN- β and iii) restoring proliferation of DM-derived MPCs with IFN-I signaling inhibitors (either IFNAR or JAK/STAT blockers).

Through investigation of the myogenic properties of MPCs, our results further defined DM and more generally IIM muscle repair as one of the cornerstones for future therapeutic approaches, and for adaptation of already in use armamentarium for such diseases.

The results obtained during my PhD program provided evidence of a cell-autonomous deficiency in IIM-derived MPCs that could clearly participate to the disease pathogenesis regarding initial steps or chronicization. However, the precise mechanisms of these alterations remain to be elucidated.

*** Further deciphering of the mechanisms responsible for intrinsic impairment of IIM-derived MPCs**

*** Following the track of IFN-I**

Confirmation of IIM derived-MPC autocrine production of IFN-I *in vitro* could be performed by SIMOA analysis of cell culture supernatant. Nevertheless, such analysis may be difficult as IFN-I quantity may be found at extremely low concentrations (attomolar values), and the potential variability of SIMOA detection results supports the need for a detailed and rigorous experimental protocols.

Another way to further define the IFN-I involvement in IIM derived-MPCs could be to perform a transcriptomic analysis of these cells.

*** To define if the cells have been imprinted by epigenetic events**

Our hypothesis is that in DM muscle, the negative feedback loop allowing to IFN-I production containment seems inefficient and replaced by a positive feedback loop: produced IFN is secreted, binds to IFNAR on pDCs and leads to IFN production, even without external stimuli and that such phenomenon is maintained over time (Liao et al., 2010). Therefore, one hypothesis could be the existence of epigenetic modifications induced by IFN *in vivo* leading to secondarily intrinsic cell dysfunction. Following this hypothesis, DM- and JDM-derived MPCs may be unable to proliferate consequently to a first IFN-I print followed by an autonomous self-sustained cellular dysfunction, leading to a defect of muscle repair that may participate to the fact that patients with IIM rarely fully recover their initial strength (Clarke et al., 1995; Bronner et al., 2006).

Accordingly, further investigations may be performed to make a link between IFN-I signaling and the epigenetic control of the expression of the myogenic program. For this purpose, several lines of research may be explored.

Our privileged avenue of research would be to evaluate the hypothetical role of HIRA, a histone chaperone complex that stands as an essential complex for the deposition of the histone variant

H3.3, a variant deposited at promoters and regulatory elements in a DNA-synthesis independent manner. It has been shown that in human fibroblasts HIRA complex localization depends on IFN signaling (Cohen et al., 2018). Upon IFN treatment, HIRA relocalizes in specific nuclear bodies called promyelocytic leukemia nuclear bodies (PML-NBs), a process that is reversible upon ruxolitinib treatment. Moreover, preliminary data indicate that HIRA is also preferentially relocated in PML-NBs in MuSCs from severe DM patients. Interestingly, histone variant H3.3 was found to be deposited at regulatory and enhancer regions of MyoD, the key myogenic regulator factor, playing a critical role for skeletal myogenesis (Yang et al., 2011)

Together data showing that IFN-I impairs muscle regeneration and above literature suggest the involvement of HIRA in a downstream pathway. The experimental perspectives could be:

- to identify the localization of HIRA in skeletal muscle biopsies of IIM patients,
- to link IFN levels in MuSCs with the extent of HIRA localization in PML-NBs,
- to assess whether ruxolitinib treatment restores a normal pan-nuclear localization of HIRA in MuSC nucleus.

*** Investigating the NF- κ B pathway**

Overexpression of MHC-I by myofibers is a hallmark of IIM histology (van der Pas et al., 2004). MHC-I gene transfer results in myogenesis impairment, notably the differentiation step both *in vivo* and *in vitro* (Pavlath, 2002). In a study evaluating muscle tissue from myositis patients and from a MHC-I transgenic mouse model of myositis, authors demonstrated the activation of the ER stress response (Nagaraju et al., 2005). The link between MHC-I overexpression and RE stress response has been discussed, as MHC-I staining in muscle fibers of myositis patients present both cell surface and sarcoplasmic reticulum pattern of reactivity, demonstrating that some MHC-I may be retained in the ER of these fibers. The ER overload response is known to activate NF- κ B pathway, with subsequent expression of pro-inflammatory cytokines and regeneration impairment. In addition, it has been demonstrated that NF- κ B negatively impacts the myogenic potential of muscle-derived stem cells (Lu et al., 2012).

To assess the involvement of NF- κ B pathway in myogenesis properties impairment of IIM-derived-MPCs, a first step could be to quantify the level of phosphorylated p65, one of the five subunits of NF- κ B family, that composes the most common form of transcriptionally active

NF- κ B. Another experiment could be to evaluate the consequences of selective pharmacological inhibition of IKK β , an upstream activator of NF- κ B, on myogenesis from IIM-derived MPCs.

*** Future directions for therapy**

From my study, the main direction for future therapy is the inhibition of JAK/STAT pathway, as JAK inhibitors are available and widely used drugs. Indeed, our results support the possibility that blocking JAK/STAT pathway has favorable effects on muscle repair. Further experimentation including larger cohort of patients remains necessary *in vitro*, and clinical research with clinical trial would be of great value.

Another interesting pathway could be the evaluation of moderate exercise as this has been reported to suppress NF- κ B signaling and activates the SIRT1-AMPK-PGC1 α axis to attenuate muscle loss (Liu et al., 2018). The notion of ‘epi’ memory of skeletal muscle and the influence of exercise on such molecular and epigenetic mechanisms enabling skeletal muscle to adapt was recently discussed (Sharples et al., 2016).

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**SCIENTIFIC PUBLICATIONS AND
COMMUNICATIONS**

Scientific publications

Gallay L, Mouchiroud G, Chazaud B. Interferon-signature in idiopathic inflammatory myopathies. **Curr Opin Rheumatol**. 2019 Nov;31(6):634-642. PMID: 31464706

Aussy A; Fréret M; Gallay L; Bessis D; Vincent T; Julien D; Drouot L; Jouen F; Joly P; Marie I; Meyer A; Sibilia J; Bader-Meunier B; Hachulla E; Hamidou M; Hue S; Charuel J-L; Fabien N; Viailly P-J; Allenbach Y; Benveniste O; Cordel N; Boyer O; OncoMyositis Study Group. The IgG2 isotype of anti-transcription intermediary factor 1-gamma autoantibodies is a biomarker of mortality in adult dermatomyositis, **Arthritis Rheumatol**. 2019 Aug;71(8):1360-1370. doi: 10.1002/art.40895. Epub 2019 Jul 8. PMID: 30896088.

Gallay L, Hot A, Petiot P, Thivolet-Bejui F, Maucort-Boulch D, Streichenberger N. Focal myositis: New insights on diagnosis and pathology. **Neurology**. 2018, PMID: 29467303

Communications

Oral communications

- Journées Neuro-musculaire PACARA (Saint Etienne, France – September 2019) “Dysfonction des cellules souches musculaires dans la dermatomyosite”
- National congress of Internal Medicine Society (Montpellier, France – June 2019) “Avancées sur le projet DM/REGEN: évaluation de la myogénèse dans la dermatomyosite”
- Global Conference On Myositis (GCOM) (Berlin, Germany – April 2019) “Myogenesis impairment in Dermatomyositis.”
- Days of the French Society of Myology (Brest, France – November 2018) “Myogenesis impairment in Dermatomyositis.”
- National congress of Internal Medicine Society (Montpellier, France – June 2018) “Etude de la myogénèse dans la Dermatomyosite”
- Internal seminar of the Institut NeuroMyoGène (Lyon, France - December 2017) “Myogenesis impairment in Dermatomyositis.”

Poster communications

- Global Conference On Myositis (GCOM) (Berlin, Germany – April 2019) “Myogenesis impairment in Dermatomyositis”.

- PhD School day (Villeurbanne, France – October 2018) “Myogenesis impairment in Dermatomyositis.”
- Annual Day of Young Researchers of the Institut NeuroMyoGène (Lyon, France – February 2019) “Myogenesis impairment in Dermatomyositis.”
- conference « Exercise, locomotion and musculoskeletal system » (Lyon, France – May 2018) “Myogenesis impairment in Dermatomyositis”

Price and gratification

2018 : Young researcher award of French society of internal medicine

2016-2019 : PHD grant : “Poste d’accueil INSERM” (36 month)