In some experiments macrophages were stimulated with the itaconate derivative 4-Octyl itaconate (4OI) at 100 μM for 2 hours, then stimulated or not with LPS for a further 24 hours. IL-1β and IL-6 cytokines were measured by ELISA. Bleomycin mouse model of skin fibrosis was employed by repeated intra dermal injections of bleomycin or sodium chloride vehicle. Skin biopsies were taken in the lesioned skin and H&E performed and fibrosis markers by qPCR was analysed.

Results: Healthy monocytes in response to LPS stimulation robustly upregulated IRG1 expression but in comparison to SSc monocytes this upregulation was significantly attenuated (n=3). Pretreatment of SSc monocytes with the itaconate derivative 4OI prior to LPS stimulation led to significantly reduced IL-1β and IL-6 secretion.

The also led to upregulation of the nr2f2 target gene Nqo1 6 fold compared to LPS treated alone. No difference in nf-kb was observed. mRNA expression of bleomycin skin found significantly downregulated nr2f2 expression in association with elevated fibrosis markers compared to vehicle control treated mice (n=3 per group).

Conclusion: Failed upregulation of IRG1 in SSc macrophages may lead to a failure of inflammatory resolution and subsequent fibrosis. The itaconate derivative 4OI could be a possible anti-fibrotic through restoration of homeostasis via upregulation of nr2f2 target genes.

Disclosure of Interests: Steven O’Reilly Employee of: I am employed by STipe therapeutics

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AB0129

MYOSITIS-SPECIFIC AUTOANTIBODIES IN CLINICAL PRACTICE: IMPROVING THE PERFORMANCES OF THE IMMUNODOT

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Background: Idiopathic inflammatory myopathies (IIM) or myositis are a group of rare autoimmune diseases that combine muscle weakness and multi-visceral damage. The discovery of IIM-specific autoantibodies (aAbs) and their associations with clinical phenotypes has improved diagnostic and classification criteria. Faced with the large number of these aAbs, multiplexed techniques have emerged. Among them, the immunodot is simple, rapid, and inexpensive, but has been several times criticized for its lack of specificity.

Objectives: Our objective was to evaluate the current interpretation criteria of the D-Tek immunodot and to propose new interpretation rules based on clinical criteria in order to improve its performances.

Methods: Were included in this retrospective study patients tested positive result on the semi-quantitative myositis/synthetase immunodot at manufacturer threshold (≥ 5 UA), for at least one of the 15 aAbs: anti-SRP, anti-NXP2, anti-TIF1, anti-SAE (1 and 2), anti-Mi2, anti-MDA5, anti-Jo1, anti-PML, anti-PL2, anti-EJ, anti-OJ, anti-KS, anti-ZO, anti-HA. Sensitivity of the immunodots was further evaluated using 60 healthy and anonymous subjects (French blood bank, Toulouse, France). The clinical diagnosis and sub-classification retained by the clinician in charge of the patient was used as a reference for attribution to the myositis/myositis-non-myositis group and sub-groups. For the myositis group, 7 groups were considered: immune-mediated necrotizing myopathy (n=4); dermatomyositis (n=6); anti-synthetase syndrome (n=36), inclusion body myositis (n=1); overlap myositis with another connective tissue disease (n=7); polymyositis (n=8); and unclassified myositis (n=6). For the non-myositis group, patients were subdivided in 4 subgroups: autoimmune or inflammatory diseases (n=72); isolated and diffuse infiltrant liver disease (n=26) not associated with other myositis criteria; other non-inflammatory myopathies (n=8) including genetic, metabolic, and toxic myopathies; and other diseases (n=36). The immunodot interpretation thresholds were evaluated both in relation to the manufacturer’s threshold, and by considering the phenotypes and clinical diagnoses using a ROC method (Youden’s index).

Results: Among 270 patients included between 01/07/2016 and 30/06/2020, 128 (47.8%) were classified as myositis (median age 58 years, 60% women, 52% DM and 28% AS) and 142 (53%) in non-myositis. Among the 15 aAbs analyzed, none were detected in the healthy control group but they were represented in both myositis and non-myositis group. Among them only 2 (anti-Jo1, anti-Mi2) predominate in the myositis group, and 1 (anti-TIF1) in the non-myositis group (Fisher’s test). As quantitative values were found different for 6 aAbs (Mann Whitney test), a clinical threshold was calculated to discriminate myositis from non-miositis groups (ROC curve) allowing to determine an odds ratio (OR). Accordingly, 4/15 (%) aAbs were found associated with myositis: anti-SRP (at 28UA: OR=3.24 95% CI [1.01-10.46], p=0.048), anti-MDA5 (at 15UA: OR=4.36; 95% CI [1.19-15.99], p=0.048), anti-Mi2 (at 3UA: OR=3.24; 95% CI [1.01-10.46], p=0.026), anti-Jo1 (at 1UA: OR=12.20; 95% CI [2.78-53.52], p<0.0001). All positive predictive values were improved by using a clinical threshold although some of them did not reach significance due to their infrequency.

Conclusion: In this retrospective work, despite missing data, the clinical phenotypes of myositis patients and their distribution according to aAbs were...
AB0130
DEREGULATION OF TFAM EXPRESSION PROMOTES MITOCHONDRIAL DAMAGE AND FIBROBLAST ACTIVATION IN SYSTEMIC SCLEROSIS
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Background: Transcription factor A, mitochondrial (TFAM) is a transcription factor with essential function in the mitochondrial homeostasis, such as mitochondrial biogenesis and mtDNA replication. Deregulation of TFAM expression has been linked to mitochondrial dysfunction. However, its role in the pathogenesis of rheumatic diseases has not been studied so far.

Objectives: We aimed to study the role of TFAM in the pathological fibroblast activation in SSc.

Methods: The expression of TFAM in SSC skin fibroblast and skin biopsies was analyzed by immunofluorescence and Western blot. The role of TFAM in fibroblast activation was investigated by TFAM knockdown in cultured fibroblasts. The role of TFAM in skin and lung fibrosis was further studied in mice with fibroblast-specific knockout of TFAM in three independent mouse models: Bleomycin-induced skin and lung fibrosis as well as TGF-βRIact-induced skin fibrosis.

Results: Dermal fibroblasts from SSc patients express lower level of TFAM in the skin and also after prolonged culture in vitro. The downregulation of TFAM in skin and fibroblasts was further studied in mice with fibroblast-specific knockout of TFAM in three independent mouse models: Bleomycin-induced skin and lung fibrosis as well as TGF-βRIact-induced skin fibrosis.

Conclusion: The expression of TFAM is decreased in SSC skin fibroblast and possibly also in skin biopsies, which is associated with decreased mitochondrial function in SSc fibroblasts. The downregulation of TFAM expression could be an early sign of alterations in mitochondrial homeostasis and a risk factor for fibrogenesis in SSc fibroblasts.

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Figure 1: A: Odd Ratio (OR) evaluated with the manufacturer threshold ≥ 5 and B: OR using Youden’s index (clinical threshold).

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AB0131
INDUCTION OF COLLAGEN CHAIN FORMATION IN RESPONSE TO FIBROTIC FACTORS IN DERMAL AND PULMONARY FIBROBLASTS
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Background: Systemic sclerosis (SSc) patients who develop pulmonary fibrosis, have an increased mortality rate.1 Excessively activated fibroblasts deposit extracellular matrix (ECM), which leads to fibrosis resulting in stiffening of the tissue in both skin and lung. Currently there is no cure for fibrosis in SSc, only drugs which can slow the fibrotic process, and there is therefore, a medical need for further understanding of the pathogenesis. Fibrosis is associated with growth factors, including tumor growth factor beta 1 (TGF-β1) and platelet derived growth factor-ab (PDGF-ab).

Objectives: We investigated how TGF-β1 and PDGF-ab stimulation affected the gene and protein expression of specific collagen chains of type I, III and VI collagen in primary healthy human dermal (DF) and pulmonary fibroblasts (PF).

Methods: The fibroblasts were grown in 0.4% fetal calf serum DMEM, ficol (to produce a crowded environment) and ascorbic acid for up to 12 days. They were stimulated with TGF-β1 [0.01 nM], PDGF-ab [3 nM] or a combination of TGF-β1 [0.01 nM] and PDGF-ab [3 nM], while non-stimulated fibroblasts served as control. ECM protein formation was assessed in supernatant from day 0, 4, 8, and 12, by ELISAs which detects the N-terminal of the pro-collagen of type I and III collagen, and the C5 domain of the α3 chain of type VI collagen. Gene expression was analyzed after 4 days and the relative gene expression was calculated based on multiple reference genes. Statistical analysis includes one-way and two-way ANOVA.

Results: TGF-β1 increased the gene expression of Col1α1 in DF (p<0.0001) and Col1α2. Col3α1 and Col6α3 in both DF (p<0.01, p<0.001, p<0.005, respectively) and PF (p<0.0001) compared to control. PDGF-ab showed no difference in gene expression of the DF but increased multiple genes in PF (p<0.01). The combination of TGF-β1 and PDGF-ab increased the gene expression of Col1α1 in DF (p<0.01), Col5α1 in PF (p<0.0001) Col1α2 and Col6α3 in both DF (p<0.05 and p<0.01) and PF (p<0.0001) compared to control. None of the stimulations lead to an increase in the Col6α2 and Col6α3. The TGF-β1 induced gene expression corresponded with increased ECM formation of type I and VI collagen from day 4 (p<0.01), and type III collagen from day 8 (p<0.05) in both DF and PF. PDGF-ab stimulation led to an increased ECM formation of type I and VI collagen in both DF and PF (p<0.01), and type III collagen in DF (p<0.01). The combination stimulation with TGF-β1 and PDGF-ab induced a corresponding increase in both gene expression and ECM formation of type I and VI collagen in both DF and PF (p<0.01). While the combination increased the ECM formation of type III collagen in both fibroblast types, the gene expression of Col5α1 were only increased in PF (p<0.0001).

Conclusion: This study demonstrates that TGF-β1 stimulation alone and in combination with PDGF-ab results in increased gene and protein expression of type I and VI collagen in both DF and PF, and additionally type III collagen in PF. However, there was a disconnect between the gene and protein expression profiles after PDGF-ab stimulation, which have to be investigated further. This study may provide new insights to the differences between fibroblast of different origin and their response to fibrotic factors.