inhibition of miR-125b. Gene ontology revealed apoptosis regulation as the main activated pathway. Apoptotic genes included BAK1, BMF and BBC3, which are part of the BCL2 apoptosis pathway and predicted targets of miR-125b. Consistent with the sequencing results, qPCR confirmed that miR-125b knockdown upregulated these genes 24, 48 and 72 hours after transfection (n=12, p<0.01 for each). BAK1 showed the strongest induction, that was also confirmed on the protein level by Western blot. Accordingly, miR-125b knockdown resulted in an increased apoptosis (at least 1.5-fold, n=10, p<0.01) compared to scrambled controls, measured by Caspase-Glo 3/7 assay 24, 48 or 72 hours post-transfection. Consistently, miR-125b overexpression decreased apoptosis (by at least 50%, n=10, p<0.01) at these time points. Cleaved caspase 3 was upregulated in anti-miR-125b transfected cells (median 2.3 fold, $Q_{1,3}$ 1.6, 4; n=10, p<0.01) confirmed by Western Blot. Annexin V live assay showed prevailing of apoptosis after miR-125b downregulation.

Conclusions: MiR-125b is downregulated in SSc skin and primary SSc dermal fibroblasts. MiR-125b downregulation increases apoptosis in dermal fibroblasts that might be a compensatory strategy against excessive fibrosis that could be used for therapeutic purposes.

Disclosure of Interest: A. Kozlova: None declared, E. Pachera: None declared, B. Maurer Grant/research support from: AbbVie, Protagen, EMDO, Novartis, German SSc Society, OPO foundation, congress support from Pfizer, Roche, Actelion. MSD. Patent licensed: mir-29 for the treatment of systemic sclerosis, A. Jüngel: None declared, J. Distler Shareholder of: 4D Science, Grant/research support from: Anamar, Active Biotech, Array Biopharma, BMS, Bayer Pharma, Boehringer Ingelheim, Celgene, GSK, Novartis, Sanofi-Aventis, UCB, Consultant for: Actelion, Active Biotech, Anamar, Bayer Pharma, Boehringer Ingelheim, Celgene, Galapagos, GSK, Inventiva, JB Therapeutics, Medac, Pfizer, RuiYi and UCB, G. Kania Grant/research support from: Bayer Pharma AG, Germany; conference support: Actelion, O. Distler Grant/research support from: Actelion, Bayer, Boehringer Ingelheim, Mitsubishi Tanabe Pharma and Roche; patent mir-29 for the treatment of systemic sclerosis licensed, Consultant for: Actelion, Bayer, BiogenIdec, Boehringer Ingelheim, ChemomAb, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, Lilly, medac, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Sinoxa and UCB

DOI: 10.1136/annrheumdis-2018-eular.2309

FRI0403 MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS IN MYOSITIS: A CENTRAL PATHOGENIC PATHWAY FROM MOUSE TO MAN

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Background: Myositides are severe diseases leading to a bedridden state and possibly death. The lack of animal model with spontaneously-occurring autoimmune myositis has hampered pathophysiological and therapeutic research. Autoimmune-prone NOD mice represent an invaluable model of type 1 diabetes (T1D). Inducible T cell co-stimulator (ICOS) is involved in germinal centre reaction and induction of helper T cell responses. We developed a unique model of myositis by invalidating the ICOS pathway in NOD mice[1]. Muscle holoproteome analysis in diseased mice together with observations of mitochondrial dysfunction in patients with dermatomyositis[2] suggest a main role of oxidative stress in disease pathogenesis.

Objectives: To determine the pathogenic role of oxidative stress and evaluate the effect of antioxidant therapy on *Icos*$^{-/-}$ NOD myositis.

Methods: Disease course was studied in *Icos*$^{-/-}$ NOD mice by grip strength, locomotor analysis and MRI. Muscle pathology was evaluated after conventional stainings, or by immuno-enzymology or immuno-histochemistry. Muscle-infiltrating cells were characterised by flow cytometry. New autoantibodies were identified in mice by serum proteomic analysis and sought for in myositis patients by ALBIA. Mouse muscle proteomic and transcriptomic analyses were performed by LC-MS/MS (Orbitrap) and RT-PCR, respectively. Muscle free radical production was assessed by EPR. Mice were treated by prednisolone (10 mg/kg/day) or N-acetylcysteine (2 g/L) in drinking water.

Results : *Icos*$^{-/-}$ NOD mice did not develop diabetes. Instead, myositis spontaneously occurred with decreased grip strength, impaired cadence, reduced print area and death around 40 wks. Pathological muscle analysis revealed necrotic myofibers and important inflammatory infiltrates ($CD4^+$ T cells, macrophages). Muscle lesions yielded MRI T2 hypersignals that regressed under steroids. $CD4^+$ T cells transferred disease to NOD.*scid* recipients. Serum proteomic analysis revealed a new autoantibody directed against a mitochondrial antigen. It was found present in ~1% individuals from a~700 myositis patient cohort. Oxydative stress was manifest in *Icos*$^{-/-}$ NOD muscle by augmented free radical production, H_2O_2 production-related atrophy, altered O_2 consumption and dysregulation of several mitochondrial genes and proteins. N-acetylcysteine partially prevented myositis or ameliorated established disease.

Conclusions: This work establishes *Icos*$^{-/-}$ NOD mice as a unique paradigm of myositis. A new autoantibody was discovered. Oxidative stress is present in diseased muscle. Antioxidant therapy is effective in a preventive or curative fashion. Together with our previous data in dermatomyositis patients[2], this work indicates the central role of mitochondrial dysfunction and oxidative stress in myositis pathogenesis and opens new perspectives for therapy.

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Acknowledgements: This work was supported by the Association Française contre les Myopathies, the European Union and Normandie Regional Council. Europe gets involved in Normandie with European Regional Development Fund (ERDF).

Disclosure of Interest: O. Boyer Grant/research support from: CSL Behring, Celogos, Consultant for: CSL Behring, Inova Diagnostics, A. Meyer: None declared, C. Boitard: None declared, C. Abad: None declared

DOI: 10.1136/annrheumdis-2018-eular.1154

FRI0404 CHARACTERISATION OF A MONOCYTES/MACROPHAGES CELL SUBSET CO-EXPRESSING BOTH M1 AND M2 PHENOTYPE MARKERS IN SYSTEMIC SCLEROSIS PATIENTS

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Background: In the pathogenesis of systemic sclerosis (SSc), the immune cell activation is an important event that includes alteration in the macrophage polarisation.[1]

Macrophages may polarise into classically activated (M1), which are characterised by the expression of specific markers such as Toll-like receptors (TLR2 and 4) and costimulatory molecules (CD80 and CD86), or alternatively activated (M2), which are characterised by the expression of specific phenotype markers such as mannose receptor-1 (CD206) and macrophage scavenger receptors (CD204 and CD163). M2 are present in the circulation and in the skin infiltrate of SSc patients (pts), where they seem to contribute to fibrosis.[2–4]

Objectives: To characterise circulating M2 monocytes/macrophages from SSc pts and healthy subjects (HSs) by their co-expression of CD204, CD163 and CD206, as well as cells expressing both M1 and M2 phenotype markers.

Methods: Fifty-eight SSc pts (54 females/4 males, mean age 63±13 years), fulfilling the new EULAR/ACR criteria for SSc, and 27 age-matched HSs were consecutively enrolled after Informed Consent was obtained. Peripheral blood was collected and the antibodies CD14-APC-Vio770 and CD45-VioGreen were used to identify the monocyte/macrophage lineage; CD204-VioBright-FITC, CD163-PE-Vio770 and CD206-PeerCP-Vio700 were used to characterise the M2 phenotype, whereas CD80-APC, CD86-VioBlue, TLR4-PE and TLR2-PE-Vio615 were used to characterise the M1 phenotype (Miltenij Biothech). Flow Cytometry analysis was performed using Navios Flow Cytometer and the related Navios analysis software (Beckman Coulter).

Results: In the $CD14^+$cell subset (monocytes), the $CD14^+CD163^+CD206^+CD204^+$cell percentage was significantly increased in SSc pts compared to HSs (p=0.02). Inside the $CD14^+CD163^+CD206^+CD204^+$monocytes/macrophages a subset of cells co-expressing also TLR4, CD80 and CD86 was detected. This mixed population (M2/M1) of cells was significantly increased in SSc pts compared to HSs (p=0.003).

At the same time, circulating monocytes/macrophages showing a full M2 phenotype and characterised as $CD204^+CD163^+CD206^+$cells were investigated independently of the expression of CD14, and they also resulted significantly increased in SSc pts compared to HSs (p<0.0001).

In the $CD204^+CD163^+CD206^+$cell subset (M2), the percentage of cells expressing also TLR4, CD80 and CD86 (M1) was significantly increased in SSc pts compared to HSs (p<0.0001).

Conclusions: These results describe for the first time a subset of circulating cells belonging to the monocyte/macrophage lineage with a mixed phenotype, which are characterised by the expression of both M1 and M2 surface markers. These

cells were observed to be increased in the peripheral blood of SSc pts compared to HSs, suggesting their possible role in the pathogenesis of the disease.

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Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2018-eular.6225

FRI0405 A NOVEL ANIMAL MODEL FOR SYSTEMIC SCLEROSIS INDUCED BY IMMUNISATION OF ANGIOTENSIN II RECEPTOR 1

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Background: Systemic sclerosis (SSc) is a complex connective tissue disease which is characterised by autoimmunity, vasculopathy and fibrosis[1]. Our recent study showed that the progression of SSc was strongly associated with the autoantibodies against angiotensin II receptor I (AT1R), suggesting a role of autoimmunity to AT1R in the pathogenesis of the disease [2]
Objectives: In this study, we aimed to investigate the role of AT1R in the pathogenesis of SSc in mice.
Methods: C57BL/6J mice were immunised with membrane extract (ME) of CHO cell overexpressing human AT1R or with ME of CHO cells as control. Serum, lung and skin samples were collected and assessed 63 days after immunisation for autoantibody production, inflammation and fibrosis, which are hallmarks for SSc.
Results: Immunisation with hAT1R induced the production of autoantibodies against the receptor in mice, and autoantibody deposition was found in the lung. Histologically, mice immunised with hAT1R showed a SSc-like disease, including perivascular infiltrates and fibrosis in the skin as well as pulmonary inflammation. The inflammation in the skin and the lung were characterised by infiltration of T- and B-cells. Furthermore, transfer of immune cells from hAT1R-immunised mice into C57BL/6J mice induced inflammation in the lung.
Conclusions: This study demonstrates that immunisation with hAT1R can induce a SSc-like disease, thus showing a pathogenic role of autoimmunity to AT1R and providng a novel mosue model for the diseases. Furthermore, this study also introduces a new immunisation strategy to generate functional autoantibodies against receptors on the cell membrane.

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Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2018-eular.1898

FRI0406 INCREASED FREQUENCY OF CIRCULATING CD163+ NON-CLASSICAL MONOCYTES IN SCLERODERMA AND ENHANCED DUAL POLARISATION TOWARDS M1 AND M2-LIKE PHENOTYPES IN MONOCYTE-DERIVED MACROPHAGES

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Background: Scleroderma (SSc) is an autoimmune connective tissue disease involving complex interactions between various cell types leading to organ-based tissue fibrosis. Emergence of the monocytes (Mo)/macrophages (Mϕ) lineage(s) as key contributors to inflammation, vascular dysfunction and scarring in scleroderma[1,2] have led to increased scrutiny of their phenotype and function.
Objectives: To determine the circulating Mo subpopulations and phenotypes of Mϕ in SSc.
Methods: PBMC were collected from healthy (HC) and SSc donors, and analysed by flow cytometry using Mo phenotypic antibodies or purified and cultured *in vitro*. For flow cytometry immunophenotyping, Mo were gated on $CD3^-CD19^-CD56^-HLA\text{-}DR^+$populations, and subsets defined by CD14, CD16, CD163 and CD206 expression. For Mϕ cultures, Mo were negatively selected from PBMCs, cultured for 7 days, and treated with IFN-γ(5 ng/ml) or IL-4(20 ng/ml) for 24 hours. Cytokine levels in the conditioned media were evaluated by MSD analyses and normalised to total protein levels.
Results: The frequency of circulating $CD163^+$ non-classical Mo ($CD14^{lo}CD16^{hi}$) was 2-fold higher in SSc patients than in HC (unpaired t-test, p=0.026). No difference was found in the frequency of $CD206^+$ monocyte subsets between HC and SSc. *In vitro*, unstimulated SSc Mϕ (M0) secreted higher levels of classically-activated pro-inflammatory (M1) and alternatively-activated pro-regenerative (M2) cytokines. Compared to HC cells, SSc Mϕ were more readily polarised towards an M1 phenotype or an M2 phenotype, when cultured in the presence of IFN-γ or IL-4, respectively. Th17 markers and MMPs were significantly increased in SSc Mϕ (table 2).

Abstract FRI0406 – Table 1. Demographics.

	Mo (flow cytometry)		Mϕ supernatant (cytokine assay)	
n	HC n=9	SSc n=10	HC n=13	SSc n=27
Age (years)	56.7±14.3	50.7±5.7	60.6±16.7	52.1±13.0
Female : Male	7:2	8:2	6:7	26:5
SSc subtype	-	dcSSc([10]	-	dcSSc([27]
Disease duration	-	≤5 years([10]	-	≤5 years(,[15]>5 year([12]

Abstract FRI0406 – Table 2. Cytokines significantly increased in SSc vs control. Unpaired t-tests, *p<0.05, **P<0.01.

Cell Population	M1 Markers	M2 Markers	Th17 Cytokines and MMP's
M0	CCL-3, CCL-4, IL-6, IL-18, IL-12, IL-15, TNF-α	CCL-1, CCL-17, CCL-20, CCL-24, IL-1Rα, YKL-40	IL-21, IL-22, IL-31, MMP1, MMP3
M0 + IFN-γ	CCL-3, CCL-4, CCL-11, CXCL8, IL-1β, IL-6, IL-18, TNF-α	CCL-1, CCL-22, CCL-24, CCL-26, IL-1Rα, YKL-40	IL-21
M0 + IL-4	CCL-3, CCL-4, IL-12, IL-18, TNF-α	CCL-17, CCL-22, CCL-24, IL-1Rα, YKL-40	IL-21, MMP3

Conclusions: Studies exploring Mo have revealed distinct populations with selective biological functions. Our observation of an increased number of $CD163^+$ non-classical Mo in SSc suggests that this subpopulation may play a key role in inflammatory-driven fibrosis and act as an important source of pro-fibrotic cytokines. This data is consistent with previous reports of elevated serum levels of CD163 and increased CD163 secretion by SSc PBMCs[3]. SSc Mϕ showed a pronounced and enhanced dual M1 and M2 polarisation basally compared to HC, indicating cells were 'primed' to undergo phenotypic polarisation. Our studies support the notion that Mϕ cytokine secretion generates a pro-fibrotic milieu in scleroderma tissues, playing a prominent role in dysregulated tissue repair in fibrosis.

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Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2018-eular.1818

FRI0407 DIPEPTIDYL-PEPTIDASE-4 (DPP4) IS A POTENTIAL NEW MOLECULAR TARGET FOR TREATMENT OF FIBROSIS

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Background: Dipeptidyl-peptidase-4 (DPP4) plays a role in tissue scaring and its inhibition leads to reduced scar formation. Its function in tissue fibrosis, however, is unknown.
Objectives: The aim of the study was to investigate the expression of DPP4 in fibrotic tissue of systemic sclerosis (SSc) patients, to characterise DPP4 positive cells, to study the mechanism of action of DPP4 in fibroblasts and to evaluate the antifibrotic effect of pharmacological and genetically inhibition of DPP4 in different preclinical models of SSc.
Methods: Expression of DPP4 in human and murine skin was analysed. Mouse fibroblasts were isolated and DPP4 positive cells properties were assessed.