inhibition of miR-125b. Gene ontology revealed apoptosis regulation as the main activated pathway. Apoptotic genes included BAK1, BMF and BCCS, which are part of the BCL2 apoptosis pathway and predicted targets of miR-125b. Consistent with the cell survival 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, Q1,3 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. Pacher: None declared, B. Maurer Grant/research support from: AbbVie, Proltag, 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, Cellaspos, Enzon, Ferring, Meriden, IB Therapeutics, Medac, Pfizer, Rucaparib, UCBB, K. Garia Grant/research support from: Bayer 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, Bio- genidec, Boehringer Ingelheim, ChemomAb, esqreare foundation, Geenentech/ Roche, GSK, Inventiva, Italfarmaco, Lilly, medac, Medimmune, Mitsubishi, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Sinova and UCBB.


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

O. Boyer1, A. Meyer2, C. Boitard3, C. Abad1. 1Inserm U1234, Normandy University, Rouen; 2EA3072, Strasbourg University Hospital, Strasbourg; 3Inserm U1016, Cochin Institute, Paris, France

Background: Myositis are severe diseases leading to a bedridden state and possibly death. The lack of animal model with spontaneously-occurring autimune 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 peripheral co-stimulation and induction of helper T cell responses. We developed a unique model of myositis by invalidating the ICOS pathway in NOD mice. Muscle holoprotein analysis in diseased mice together with observations of mitochondrial dysfunction in patients with dermatomyositis 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, whereas CD80-APC, CD86-VioBlue, TLR4-PE and TLR2-PE-Vio615 were used to characterise circulating M2 monocytes/macrophages from NOD pts and healthy subjects (HSs) by their co-expression of CD204, CD163, and CD68, as well as cells expressing both M1 and M2 phenotype markers. Methods: Fifty-eight NOD 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-Vio-Bright-FITC, CD163-Pe-Vio770 and CD206-Pe-Cy5-Vio700 were used to characterise the M2 phenotype, whereas CD80-APC, CD86-VioBlue, TRL4-PE and TLR2-Vio615 were used to characterise the M1 phenotype (Milenyi Biotech). 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+monocytes/macrophages a subset of cells co-expressing also TRL4, CD80 and CD68 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).

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.

REFERENCES:

Disclosure of Interest: None declared


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

X. Yue1, F. Petersen1, X. Wang1, H. Heidecke2, G. Riemeckasten2, X. Yu1.
1Research center borstel, Borstel; 2Celltrend GmbH, Luckenwalde; 3Department of Rheumatology, University hospital lübeck, lübeck, Germany

Background: Systemic sclerosis (SSc) is a complex connective tissue disease which is characterised by autoimmunity, vasculopathy and fibrosis. Our recent background 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.

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 cells as control, and B-cells. Furthermore, transfer of immune cells from hAT1R-immunised mice against the receptor in mice, and autoantibody deposition was found in the lung. Histologically, mice immunised with hAT1R showed a SSc-like disease, including inflammatory-driven fibrosis and act as an important source of pro-fibrotic cytokines in SSc.

Conclusions: This study demonstrates that immunisation with hAT1R can induce a SSc-like disease, thus showing a pathogenic role of autoimmunity to AT1R and providing a novel mouse model for the diseases. Furthermore, this study also introduces a new immunisation strategy to generate functional autoantibodies against receptors on the cell membrane.

REFERENCES:

Disclosure of Interest: None declared


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

A. Tam1, L. Reinke-Breen2, G. Trujillo2, S. Xu1, C. P. Denton1, D. J. Abraham1, G. Jarai1, V. H. Ong1. 1Centre for Rheumatology and Connective Tissue Disease, UCL, London, United Kingdom; 2Discovery Biology, Fibrotic Diseases, Bristol-Myers Squibb, Pennington, United States

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 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 CD3CD19CD56 HLA-DR+populations, and subsets defined by CD14, CD16, CD163 and CD206 expression. For Mo cultures, Mφ 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+CD16+) was 2-fold higher in SSc patients than in HC (unpaired t-test, p=0.028). No difference was found in the frequency of CD206+ monocyte subsets between HC and SSc. In vitro, unmaturated SSc Mo (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.

<table>
<thead>
<tr>
<th></th>
<th>HC n=9</th>
<th>SSc n=10</th>
<th>HC n=13</th>
<th>SSc n=27</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>56.7±14.3</td>
<td>50.7±5.7</td>
<td>60.6±16.7</td>
<td>52.1±13.0</td>
</tr>
<tr>
<td>Female : Male</td>
<td>7:2</td>
<td>8:2</td>
<td>6:7</td>
<td>26:5</td>
</tr>
<tr>
<td>SSc subtype</td>
<td>-</td>
<td>-</td>
<td>dCSSc10</td>
<td>-</td>
</tr>
<tr>
<td>Disease duration</td>
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<td>-</td>
<td>≤5 years10</td>
<td>-</td>
</tr>
</tbody>
</table>

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 levels of CD163 and increased CD163 secretion by SSc PBMCs. SSc Mo 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 Mo cytokine secretion generates a pro-fibrotic milieu in scleroderma tissues, playing a prominent role in dysregulated tissue repair in fibrosis.

REFERENCES:

Disclosure of Interest: None declared


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


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.

REFERENCES:

Disclosure of Interest: None declared