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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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 fibrosis¹. 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 disease².

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 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.

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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-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 PBMCs³. 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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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 scarring 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.

Pulmonary fibrosis was induced by bleomycin in DPP4 knockout (KO) mice and wildtype littermates. Fibrosis of the lungs was additionally evaluated by computer tomography scans (CT). Two oral DPP4 inhibitors were tested in two concentrations in bleomycin-induced skin fibrosis and in sclerodermatous chronic graft-versus-host disease (scl-cGVHD) model. Antiinflammatory effects of DPP4 inhibition were assessed by CD45 staining of fibrotic and non-fibrotic mouse tissue upon DPP4 inhibition. Moreover, chimeric mice were generated by transplanting bone marrow from DPP4-KO mice in WT-littermates (DPP4→WT) and vice versa (WT→DPP4) and fibrosis was by intratracheal injections of bleomycin.

Results: DPP4 positive fibroblasts were increased in fibrotic skin of SSc patients and also in murine models of fibrosis. DPP4 expression is induced by TGF- β in an Erk-dependent manner. DPP4-positive fibroblasts strongly express stress fibres after TGF- β stimulation and released increased amounts of collagen. Mechanistically, inhibition of DPP4 selectively interferes with the TGF- β induced activation of ERK signalling, but does not inhibit TGF- β induced SMAD signalling, or other non-canonical TGF- β pathways involving Fra2, c-Jun, p38, Akt or STAT3. Furthermore, pharmacological inhibition of DPP4 reduced the release of collagen and the expression of myofibroblast markers. DPP4-KO mice are less sensitive to bleomycin-induced pulmonary fibrosis as shown by milder changes on CT, reduced Ashcroft scores and reduced hydroxyproline content. DPP4-KO mice also show reduced skin fibrosis upon bleomycin challenge. Moreover, treatment with DPP4 inhibitors demonstrated potent antifibrotic effects in bleomycin-induced skin fibrosis and experimental scl-cGVHD mouse model. Treatment with DPP4 inhibitors also reduced leukocyte infiltrations into the skin. The extent of pulmonary fibrosis of DPP4→WT was comparable to that of WT→WT control mice. Fibrosis was strongly ameliorated in WT→DPP4 mice and results were comparable to that of DPP4→DPP4 mice, characterising resident cells such as fibroblasts as major target cells for the antifibrotic effects of DPP4 inhibitors.

Conclusions: DPP4 characterises an activated subpopulation of fibroblasts in SSc. Moreover, inhibitors of DPP4 show a significant anti-fibrotic effect in several mouse models of SSc in well tolerated doses. These results may have direct translational implications as DPP4 inhibitors are already in clinical use for diabetes.

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FRI0408 INVARIANT NATURAL KILLER T CELLS IN SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is a rare and heterogeneous immune-mediated disease. Its complex pathogenesis remains incompletely understood. Invariant natural killer T (iNKT) cells have been discussed in several autoimmune diseases¹. They recognise selected endogenous as well as synthetic glycolipids like α -galactosylceramide by their TCR with high affinity and when activated, they typically release high amounts of pro- and/or anti-inflammatory cytokines².

Objectives: To evaluate numbers and function of invariant natural killer T cells in patients with systemic sclerosis and analyse their correlation with disease parameters.

Methods: Human iNKT cells from 91 patients with SSc and 23 healthy controls were analysed by flow cytometric analysis. Their proliferative capacity and cytokine production was investigated following activation with CD1d ligand α -GalCer.

Results: We observed an absolute and relative decrease of iNKT cells in patients with SSc (0.036 iNKT cells/ 10^6 lymphocytes) compared with healthy controls (0.159 iNKT cells/ 10^6 lymphocytes, $p < 0.001$). We could also demonstrate that iNKT cells from patients with SSc showed a significantly decreased expansion capacity upon stimulation with α -GalCer. Interestingly, there was no difference concerning the subtype of SSc, disease severity or whether patients received immunosuppressive drugs.

Conclusions: iNKT cells are deficient and functionally impaired in patients with SSc. Therefore, adoptive transfer strategies using culture-expanded iNKT cells or administration of glycosphingolipids such as α -GalCer could be a novel approach to treat SSc patients.

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FRI0409 MYOSITIS SPECIFIC ANTI-HISTIDYL TRNA SYNTHETASE (HISRS) AUTOANTIBODIES DISPLAY HIGH REACTIVITY AGAINST HISRS CONFORMATIONAL EPITOPES AND ASSOCIATE WITH LUNG AND JOINT INVOLVEMENT

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Background: Autoimmune myositis (rheumatic muscle inflammation) associated with interstitial lung disease (ILD) and arthritis is strongly correlated with the presence of anti-histidyl tRNA synthetase (HisRS) autoantibodies.¹

Objectives: The aims of this study were to investigate: 1) myositis IgG reactivity against HisRS conformational epitopes; and 2) association between clinical manifestations and anti-HisRS reactivity profiles.

Methods: Serum IgG was isolated using a protein G affinity column (from 25 anti-HisRS negative (-) and 19 anti-HisRS positive (+) myositis sera and 24 age/gender matched healthy controls, HC). Autoantibody reactivity was tested by in house ELISA developed against HisRS full-length protein and three HisRS conformational epitopes (WHEP domain – localised in the N-terminal; HisRS without WHEP (HisRS_WHEP); and ABD – anticodon-binding domain located in the C-terminal). Correlations between diagnosis, clinical manifestations and anti-HisRS IgG reactivity were evaluated.

Results: HisRS(+) myositis IgG displayed stronger reactivity against full-length HisRS and HisRS_WHEP (median 372 ng/mL and 334 ng/mL, respectively), compared to WHEP and ABD (6.38 and 6.48 ng/mL). The strongest anti-full-length HisRS reactivity (>371 ng/mL) was detected in patients presenting ILD (10/10 of patients), arthritis (6/10) and polymyositis diagnosis (PM 9/10), in comparison to patients with low anti-HisRS reactivity (<23 ng/mL, ILD – 5/6; arthritis – 3/6; PM – 5/6) or no anti-HisRS reactivity (ILD – 10/28; Arthritis – 8/28; PM – 15/28). On the contrary, myositis patients displaying no anti-HisRS reactivity were largely diagnosed with DM (11/28), skin rash (11/28) and dysphagia (6/28) when compared to patients with the highest anti-full-length HisRS reactivity (1 out of 10 patients was diagnosed with DM, skin rash or dysphagia). Similar associations were observed between the degree of anti-HisRS_WHEP, anti-WHEP or anti-ABD reactivities and manifestations of ILD, arthritis, skin rash or dysphagia, and DM or PM diagnosis. No anti-HisRS reactivity was detected in the HC group.

Abstract FRI0409 – Figure 1. Anti-full-length HisRS IgG reactivity in myositis patients diagnosed with either polymyositis (PM) or dermatomyositis (DM), and presenting manifestations of interstitial lung disease (ILD), arthritis, skin rash and dysphagia. Numbers represent the patients included in the analysis.

Conclusions: This study provides evidences for a possible underlying role of anti-HisRS autoantibodies in the pathogenesis of myositis with interstitial lung disease and joint involvement.

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