Conclusions: In the present short-term study (24 and 48 hours), no significant effects at qRT-PCR resulted after CTLA4-Ig treatment of cultured SSc SFs. The result might arise from a limited expression of CD86, as consequence of a retained advanced differentiation of the the SSc fibroblasts. On the contrary, a significant reduction of CD86 expression on HSs fibroblasts treated with CTLA-4Ig was observed.

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FRI0418 IN VITRO EFFECTS OF CTLA4-IG TREATMENT ON CULTURED FIBROCYTES FROM SYSTEMIC SCLEROSIS PATIENTS

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Background: Circulating fibrocytes (CFs) are progenitor cells derived from bone marrow, expressing markers of both hematopoietic cells (CD45, MHC class II) and stromal cells (collagen I and III), together with the chemokine receptors, which regulate their migration into inflammatory lesions (CXCR4, CCR2, CCR7).[1] CFs can migrate into SSc-affected tissues and can differentiate into fibroblasts/myofibroblasts.[2] CFs express the CD86 (B7.2) costimulatory molecule and the adhesion molecules CD11a, CD54, ICAM-1, and CD58. The fusion molecule CTLA4-Ig interacts with CD86 and can downregulate the target cell.[3]
Objectives: To study the *in vitro* effects of CTLA4-Ig on cultured CFs.
Methods: CFs were obtained from the peripheral blood samples of 8 "limited" cutaneous SSc patients (treated only with vasodilators, mainly cyclic prostanoids) and from 4 healthy subjects (HSs). CFs were characterised by fluorescence-activated cell sorter analysis (FACS), at basal time (T0) and after 8 culture days (T8), for CD45, collagen type I (COL I), CXCR4, CD14, CD86, and HLA-DRII expression. T8-cultured CFs were treated for 3 hours in the absence or in the presence of CTLA4-Ig (10, 50, 100 and 500 micrograms/ml). Quantitative real-time polymerase chain reaction (qRT-PCR) for CD86, COL I, IL1b, TGFb, αSMA, S100A4, CXCR2, CXCR4, CD11a were performed. The statistical analysis was carried out by the nonparametric Mann-Whitney U test. Skin samples for fibroblast (SFs) cultures were obtained from the same patients after EC and patient informed consent.
Results: At qRT-PCR, T8-SSc fibrocytes, in the absence of CTLA4-Ig treatments, showed higher CD86 expression levels compared to HSs fibrocytes. Similarly also αSMA, S100A4, TGFb and COL I gene expression resulted higher in SSc fibrocytes compared to HSs. After CTLA4-Ig treatments, only in SSc fibrocytes, the αSMA and COL I gene expression resulted significantly decreased ($p<0.01$, $p<0.05$), whereas the gene expression for S100A4 resulted significantly increased ($p<0.01$), compared to untreated fibrocytes. Interestingly, in skin fibroblasts from the same SSc patients, the CD86 gene expression was found to be very low, compared to CFs.
Conclusions: Circulating fibrocytes from patients affected by limited cutaneous SSc seem to be responsive and downregulated after *in vitro* CTLA4-Ig treatments, suggesting a possible antifibrotic effect on progenitor cells before their final homing and differentiation in active myofibroblasts. Fibroblasts from the same patient do not show the same expression of target molecules and reactivity to CTLA4-Ig.

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FRI0419 INTRAVENOUS IMMUNOGLOBULINS PREVENTS EXPERIMENTAL FIBROSIS IN A MURINE MODEL OF SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterised by an extensive multi-organs fibrosis. Immunosuppressants are effective in some extent but their incomplete efficacy is hampered by a higher infection risk. Intravenous immunoglobulins (IVIG) have a good safety profile, exhibit immunomodulatory and antifibrotic properties and hence could be a relevant treatment for SSc.
Objectives: The purpose of this study was to investigate the effects of IVIG in an experimental model of SSc.
Methods: SSc was induced in 6 weeks old Balb/c mice by subcutaneous injections of HOCl five days a week during six weeks (n=20), whereas control mice received subcutaneous injections of PBS (n=20). Human IVIG was administrated intravenously by single retro-orbital injection at a dose of 2 g/kg the first day of HOCl administration (n=20). A control group received an injection of 2% Maltose (n=20). Skin thickness was assessed during the protocol until the sacrifice (day 42). Skin tissues were collected in 4% PFA and processed for histological analysis. Dermal thickness was measured by performing a May-Grünwald–Giemsa staining of 4 μm skin sections; collagen deposition was assessed by performing a Picrosirius red-staining and quantified by using a colour deconvolution method. In addition, immunostaining of skin sections was performed in order to evaluate the α-smooth muscle actin (α-SMA) expression. Frozen skin tissues were analysed to also assess the mRNA expression of main inflammatory and pro-fibrotic genes (by quantitative reverse transcription polymerase chain reaction). Collagen deposition was also evaluated by measuring the content of hydroxyproline in 10 mg of frozen tissue.
Results: Mice exposed to HOCl developed a diffuse cutaneous SSc with higher dermal thickness compared to the PBS group. IVIG significantly reduced dermal thickness and collagen deposition in HOCl-receiving mice. The amount of α-SMA positive cells evaluated by immunofluorescence was reduced in the HOCl treated mice receiving IVIG. mRNA expression profile of various markers of fibrosis (fibronectin, TGFβ) or inflammation (TNFα, IL-1β, IL-6) were also significantly decreased in the skin of HOCl mice treated with IVIG compared to HOCl-treated mice receiving 2% maltose.
Conclusions: These results demonstrate the efficacy of IVIG in preventing experimental fibrosis in a HOCl murine model of SSc.
Disclosure of Interest: None declared

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FRI0420 ALTERED TRANSCRIPTOME OF CIRCULATING CD14+ MONOCYTES IN SYSTEMIC SCLEROSIS

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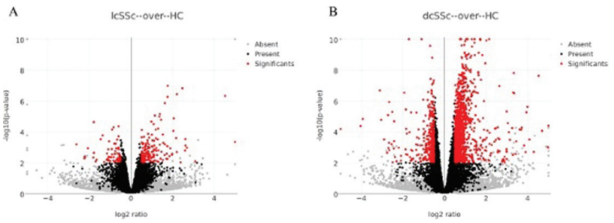
Background: Previous studies indicated monocyte-derived cells as important players in the development of multiple organ fibrosis. Although changes in monocyte's phenotypes as well as an increased infiltration into fibrotic organs were reported in systemic sclerosis (SSc), the detailed role of these cells in multi-organ fibrogenesis remains unclear.
Objectives: We aimed to characterise a contribution of circulating $CD14^+$ monocytes in the disease course in limited cutaneous (lc) and diffuse cutaneous (dc) SSc.
Methods: $CD14^+$ monocytes were isolated from peripheral blood of lcSSc (n=5, age=54.4±6.7), dcSSc patients (n=5, age=51.8±7.2) and age- and sex-matched healthy controls (HC) (n=5, age=50.8±9.7). Total RNA was isolated and polyA libraries were prepared using TruSeq Stranded mRNA kit. Next Generation Sequencing was performed using Illumina HiSeq 4000 platform. Differentially expressed genes were computed using DeSEQ2 algorithm. Principal Component Analysis (PCA) was accomplished as well as pathway analysis using Metacore software. mRNA levels of top targets were confirmed by qPCR.

Results: We detected 1440 differentially expressed genes between dcSSc vs HC and 225 between lcSSc and HC respectively ($p \leq 0.01$; log2 ratio ≥ 0.5, figure 1). Among those, in dcSSc 1076 were upregulated (e.g. MMP9, IL1R2, FLT3, MIF, TLR9) and 364 were downregulated (e.g. TGFBR1, CD44, CD244, HLA-DRA, HLA-G). In lcSSc 160 transcripts were upregulated (e.g. CCL2, WNT5B, MMP17) and 65 were downregulated (e.g. KLF11, IRAK2). We identified 123 commonly deregulated genes between SSc subgroups (e.g. CCL3, CD14, IL27, MMP17). Principal component analysis showed close clustering within SSc subgroups and clear separation from healthy controls. Pathway analysis revealed alterations in several biological processes important in fibrogenesis including antigen presentation, MIF-induced immune responses, TGF-β, NOTCH and WNT signalling pathways. qPCR analysis further confirmed differences in gene expression on mRNA level (n HC=8, n SSc=25, $p \leq 0.05$).



Abstract FRI0420 – Figure 1. Volcano plots representing differently expressed genes in lcSSc vs HC (A) and dcSSc vs HC (B). Red dots stand for significantly deregulated transcripts ($p \leq 0.01$; log2 ratio ≥ 0.5).

Conclusions: To our knowledge, this is the first global transcriptome analysis of peripheral blood $CD14^+$ monocytes in SSc. Our results suggest an initial activation of monocytes in peripheral blood, which might be further translated into novel cellular biomarker of the disease and potentially used for distinguishing between responders and non-responders to a novel treatment in future clinical trials.

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FRI0421 TOLL LIKE RECEPTOR-7/8 ACTIVATION EXACERBATES MURINE EXPERIMENTAL AUTOIMMUNE MYOSITIS

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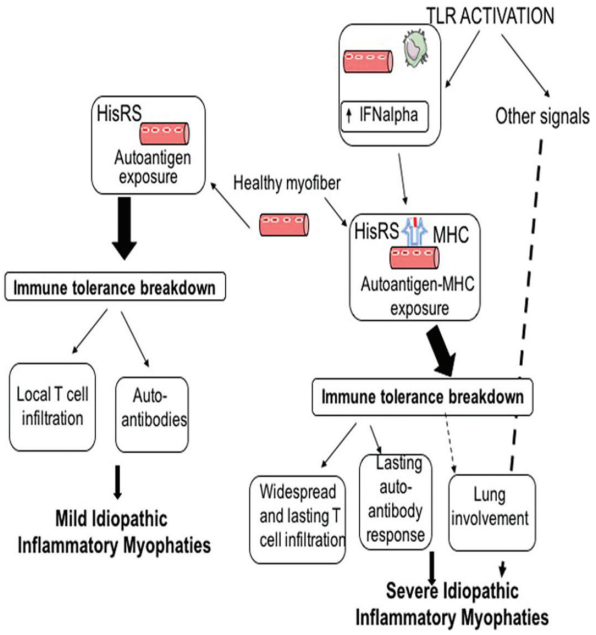
Background: Type I interferon (IFN)-regulated proteins are upregulated in muscle and skin tissues of patients with idiopathic inflammatory myopathies (IIM).[1] Type I IFN induction might rely upon the activation of toll-like receptors (TLRs).[2] Specifically, TLR-7/8 is indeed upregulated in infiltrating leukocytes and muscle tissue of IIM patients.[3]

Objectives: To investigate whether the activation of TLR-7/8 and type I IFN influences the natural history of the IIM.

Methods: Experimental autoimmune myositis (EAM) was induced by injection of the amino-terminal portion of the murine Histydil t-RNA synthetase (HisRS). Disease activity was compared in the presence or absence of the TLR-7/8 agonist R848 in wild-type mice and in mice that failed to express the IFNab receptor (IFNabR null).

Results: EAM induced by a single intramuscular immunisation with HisRS spontaneously abated after 7–8 weeks. In contrast, the levels of anti-HisRS autoantibodies, endomysial/perimysial leukocyte infiltration and myofiber regeneration persisted until the end of the follow-up period (22 weeks after immunisation) in mice immunised with HisRS in the presence of R848. Myofiber MHC class I molecules were detectable in HisRS +R848 immunised mice only. Muscle MHC expression occurred in parallel with leukocyte infiltration. Type I IFN was necessary for the prolonged autoantibody response to occur and for the spreading of the autoimmune response, as demonstrated using IFNabR null mice.



Abstract FRI0421 – Figure 1. Role of TLR7/8 activation in HisRS-induced myositis

Conclusions: TLR7/8 activation is needed to induce and maintain a systemic autoimmune response against the skeletal muscle. This EAM model reproduces many characteristics of human IIM and may represent a tool for pre-clinical studies.

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FRI0422 ROLE OF THE PROLYL 3-HYDROXYLASE LEPREL1 IN FIBROSIS

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Background: Three prolyl 3-hydroxylase enzymes, *LEPRE1, LEPREL1 and LEPREL2*, are known to modify prolines in certain sequences in the C-terminal helical region of the polypeptide chains of procollagens converting them to 3-hydroxyproline residues. This modification appears to facilitate correct alignment of the chains in forming the triple helical domains of the procollagen molecules prior to secretion. Increased deposition of triple helical collagen and other extracellular matrix (ECM) proteins by activated fibroblasts underlies pathological fibrosis in systemic sclerosis (SSc), and may be dependent on prolyl 3-hydroxylase activity as a rate-limiting step.

Objectives: The objectives of this study were: 1) to screen candidate genes with large first introns containing regulatory elements for association with systemic sclerosis (SSc) through copy number variation (CNV), 2) to study the lead candidate gene *LEPREL1* further, as a fibrosis-related factor in genetically modfied mice subject to bleomycin induced skin fibrosis, 3) to determine the levels of