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 SSc fibroblasts. On the contrary, a significant reduction of CD86 expression on HSs fibroblasts treated with CTLA4-Ig was observed.

REFERENCES:

- [1] Asano Y. J Dermatol 2017. Epub ahead of print. [Review].
- [2] Hin ZB, Phan SH, et al. Am J Pathol 2007;170:1807–16.
- [3] Cutolo M, et al. Arthritis Res Ther 2009;11:176–85.

Acknowledgements:

Disclosure of Interest: M. Cutolo Grant/research support from: BMS, Actelion, Celgene, Boehringer, P. Montagna: None declared, S. Soldano: None declared, A. C. Trombetta: None declared, P. Contini: None declared, B. Ruaro: None declared, A. Sulli: None declared, S. Scabini: None declared, E. Stratta: None declared, R. Brizzolara: None declared

DOI: 10.1136/annrheumdis-2018-eular.5244

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

M. Cutolo¹, P. Montagna¹, S. Soldano¹, A.C. Trombetta¹, B. Ruaro¹, P. Contini², A. Sulli³, S. Scabini³, E. Stratta³, R. Brizzolara¹. ¹Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, IRCCS Policlinic San Martino, Genoa; ²Division of Clinical Immunology, Department of Internal Medicine, University of Genoa, IRCCS Policlinic San Martino; ³Oncologic Surgery, Department of Surgery, IRCCS Policlinic San Martino, Genoa, Italy

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.

REFERENCES:

- [1] Bucala R. Mol Med 2015;2:S3–5.
- [2] Keeleya EC, et al. The International Journal of Biochemistry & Cell Biology 2010;42:535–42.
- [3] Cutolo M, et al. Arthritis Res Ther 2009;11:176–85.

Disclosure of Interest: M. Cutolo Grant/research support from: BMS, Actelion, Celgene, Boehringer, P. Montagna: None declared, S. Soldano: None declared, A. C. Trombetta: None declared, B. Ruaro: None declared, P. Contini: None

declared, A. Sulli: None declared, S. Scabini: None declared, E. Stratta: None declared, R. Brizzolara: None declared

DOI: 10.1136/annrheumdis-2018-eular.5173

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

M.-M. Farhat^{1,2,3}, S. Specia^{1,2,3}, M. Jendoubi^{1,2,3}, C. Hauspie^{1,2,3}, T. Guerrier^{1,2,3}, V. Sobanski^{1,2,3}, S. Sanges^{1,2,3}, E. Hachulla^{1,2,3}, G. Lefevre^{1,2,3}, M. Labalette^{1,4}, S. Dubucquoi^{1,4}, D. Launay^{1,2,3}. ¹Univ. Lille, INSERM U995 LIRIC – Lille Inflammation Research International Center; ²Centre National de Référence Maladies Systémiques et Auto-immunes Rares (Sclérodémie Systémique); ³CHU Lille, Département de Médecine Interne et Immunologie Clinique; ⁴CHU Lille, Institut Immunologie, Lille, France

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 administered 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

DOI: 10.1136/annrheumdis-2018-eular.7171

FRI0420 ALTERED TRANSCRIPTOME OF CIRCULATING CD14+ MONOCYTES IN SYSTEMIC SCLEROSIS

M. Rudnik¹, M. Stellato¹, P. Blyszczuk^{1,2}, O. Distler¹, G. Kania¹. ¹Center of Experimental Rheumatology, Department of Rheumatology, University Hospital Zurich, Zurich, Switzerland; ²Department of Clinical Immunology, Jagiellonian University Medical College, Krakow, Poland

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.