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 results 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 CD68 expression on HSs fibroblasts treated with CTLA-4lg was observed.

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FRIO418

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

M. Cutolo1, M. Montagna1, S. Soldano1, A.C. Trombetta1, B. Ruaro1, P. Contini1, A. Sulli1, S. Scabini1, E. Stratta1, R. Brizzolara1. 1Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, IRCCS Polyclinic San Martino, Genoa; 2Division of Clinical Immunology, Department of Internal Medicine, University of Genoa, IRCCS Polyclinic San Martino; 3Oncologic Surgery, Department of Surgery, IRCCS Polyclinic 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). CFs can migrate into SSC-affect ed tissues and can differentiate into fibroblasts/myofibroblasts. CFs express the CD68 (B7.2) costimulator 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.

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, CD68, and HLA-DR/I 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 CD68, COL I, IL1b, TGFb, oSMA, 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 CD68 expression levels compared to HSs fibrocytes. Similarly also oSMA, S100A4, TGFb and COL I gene expression resulted higher in SSC fibrocytes compared to HSs. After CTLA4-Ig treatments, only in SSC fibrocytes, the oSMA 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 CD68 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 anti fibrotic 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 CTLA-4lg.

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FRIO420

ALTERED TRANSCRIPTOME OF CIRCULATING CD14+ MONOCYTES IN SYSTEMIC SCLEROSIS

M. Rudnik1, M. Stelato1, P. Blyszczuk1, 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±9,7), 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 compared 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.

Disclosure of Interest: None declared.