SAT0301
CIRCULATING FIBROCYTES FROM SYSTEMIC SCLEROSIS PATIENTS AS POSSIBLE TARGET OF CTLA-4G TREATMENT: AN IN VITRO STUDY

S. Soldano1, P. Montagna1, S. Paolino1, E. Alessandri1, C. Pizzorni1, G. Pacini1, F. Goeggan2, A. Sulii3, C. Scheneone4, V. Smith5, M. Cutolo1, 2
Research Laboratory and Academic Division of Clinical Rheumatology, Dept. Internal Medicine, University of Genova, Genova, Italy; 2Department of Rheumatology, Ghent University Hospital; Department of Internal Medicine, Ghent University; Unit for Molecular Immunology and Inflammation, VIB Inflammation Research Center (IRC), Ghent, Italy

Background: muscle actin (aSMA) cells involved in the overproduction of extracellular matrix proteins, primarily fibronectin (FN) and type I collagen (COL1) at the level of damaged tissues (1). These cells may originate from different cell types including fibroblasts, endothelial and epithelial cells, and fibrocytes (1). Circulating fibrocytes are bone marrow progenitor cells expressing specific markers of hematopoietic (CD34, CD45, and MHC class II) and stromal cells (COL1 and COL3), chemokine receptors (CCR2, CCR7), and CXCR4 (2). CXCR4 regulates fibrocyte migration into injured tissues allowing their differentiation into fibroblasts/myofibroblasts (2).

In vitro, fibrocytes differentiate from circulating CD4+monocytes showing an antigen-presenting capability through the expression of HLA-DR and costimulatory molecule CD86 (2). CTLA-4g fusion protein (abacatumab) interacts with CD86 on cell surface of antigen presenting cells (APCs), such as macrophages and endothelial cells (3).

Objectives: To investigate the possible effect of CTLA-4g treatment on cultured human fibrocytes and skin fibroblasts isolated from the same systemic sclerosis patients (SSc pts).

Methods: Fibrocytes isolated from the peripheral blood mononuclear cells of SSC pts and healthy subjects (HSs) were cultured on fibronectin-coated plates in DMEM at 20% of FBS; for further 8 days (T8) to allow their complete differentiation. Differentiated fibrocytes were maintained in growth medium or treated with CTLA-4g at different concentrations (10, 50, 100, and 500ng/ml) for 3 hours. Fibrocytes were isolated from the skin biopsies of the same patients and HSs, cultured until the 3th passage in RPMI at 10% FBS and then treated with CTLA-4g for 24 and 48 hours. Fibrocytes were characterized as CD45+CXCR4+COL1+and the expression of CD86 was assessed by flow cytometry and Western blotting.

Results: Treatment with CTLA-4g for 3 hours significantly downregulated aSMa and COL1 gene expression in cultured SSC fibroblasts at T8 (p<0.01, p<0.05 vs. untreated fibrocytes), whereas no modulatory effect was observed on the TGFB- and TGF-β1-mediated expression. In cultured SSC skin fibroblasts, CTLA-4g did not induce any significant effect on CD68, TGFβ1, COL1 and FN gene expression as well as COL1 and FN protein synthesis, both after 24 and 48 hours. Of note, these cultured SSC skin fibroblasts showed a low expression of CD86.

Conclusion: Due to their high expression of CD86, circulating fibrocytes seem to be more responsive to CTLA-4g treatment than the skin fibroblasts isolated from the same SSC patient.

References:

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SAT0302
INNATE LYMPHOID CELLS INDUCE A FIBROTIC PHENOTYPE OF FIBROBLASTS

S. Weber1, C. G. Anchang1, S. Rauber2, M. Luber2, M. G. Raimondi1, Y. Ariza2,3, A. Rius Rigau4, A. Kreuter5, G. Schett6, J. Distler6, A. Ramming1, Friedrich-Alexander University (FAU) Erlangen-Nürnberg and Universitätsklinikum Erlangen, Department of Internal Medicine 3 - Rheumatology and Immunology, Erlangen, Germany; 1Friedrich-Alexander University (FAU) Erlangen-Nürnberg and Universitätsklinikum Erlangen, Department of Internal Medicine 3 - Rheumatology and Immunology, Erlangen, Germany; 2Ono Pharmaceutical Co., Ltd., Research Center of Specialty, Research Department, Osaka, Japan; 3HELIOS St. Elisabeth Klinik Oberhausen, University Witten-Herdecke, Department of Dermatology, Venerology and Allergology, Oberhausen, Germany

Background: Fibrotic diseases are characterized by excessive extracellular matrix production as a result of immune-mediated permanent fibroblast activation. Inactive lymphoid cells type II (ILC2) are an only recently discovered cell type involved in barrier integrity and tissue homeostasis. There is upcoming evidence that ILC2s play a central role in mediating fibrotic diseases.

Objectives: The aim of the study was to further elucidate the role of ILC2s in fibrotic tissue remodelling and fibroblast activation.

Methods: Skin biopsies of patients with systemic sclerosis (SSc) or scleroderma-matous chronic graft versus host disease (ssGVHD) as well as lung biopsies of patients with idiopathic pulmonary fibrosis (IPF) were analyzed by immunofluorescence (IFA)-staining (ssILC2) and single cell RNA-sequencing (ssRNA-seq) was performed on ILC2s from fibrotic skin and lung of bleomycin-challenged mice. Further characterization of ILC2 phenotypes in fibrosis models was done by flow cytometry.

In vitro culture of fibroblasts and ILC2s was used to study cellular interaction and fibroblast activation. Quantitative realtime-PCR, western blot, IF staining and ELISA were used as readouts.

Results: Two different subtypes of ILC2s were found in skin of SSc and ssGVHD patients as well as in lungs of IPF patients with one subgroup being particularly increased in fibrotic tissue. Single cell RNA-sequencing confirmed the existence of two major populations of ILC2s in experimental fibrosis. One subtype showed features of immature ILC2 progenitors and was actively recruited from the bone marrow during fibrotic tissue remodeling. The other ILC2 subset was highly activated and expressed pro-fibrotic cytokines. These pro-fibrotic ILC2s directly interacted with fibroblasts in a cell contact dependent manner. Senaphorin 4A (SEMA4A) expressed by ILC2s bound to Plexin D1 (PLXND1) on fibroblasts. This interaction resulted into fibrotic imprinting with high expression levels of the transcription factor PU.1 which was recently described as central regulator of the pro-fibrotic gene expression program (Wohlfahrt et al. 2019). Signaling through Jagged 1 (JAG1) and Notch receptor 2 (NOTCH2) was identified as a second mechanism of interaction between fibroblasts and ILC2s. JAG1 expressed by fibroblasts activated NOTCH2 signaling in ILC2s which emp- hanced the secretion of pro-fibrotic cytokines and matrix production. ILC2s incorporating a vicious circle of fibrotic tissue remodelling. As ILCs are still not accessible as therapeutic targets these results might contribute to the development of new strategies for anti-fibrotic therapies.

References:

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