SAT0301

CIRCULATING FIBROCYTES FROM SYSTEMIC SCLEROSIS PATIENTS AS POSSIBLE TARGET OF CTLA4-IG TREATMENT: AN IN VITRO STUDY

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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 CD14⁺ monocytes showing an antigen-presenting capability through the expression of HLA-DR and costimulatory molecule CD86 (2). CTLA4-Ig fusion protein (abatacept) interacts with CD86 on cell surface of antigen presenting cells (APCs), such as macrophages and endothelial cells (3,4).

Objectives: To investigate the possible effect of CTLA4-Ig 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 CTLA4-Ig at different concentrations (10, 50, 100, and 500 µg/ml) for 3 hours. Fibroblasts were isolated from the skin biopsies of the same patients and HSs, cultured until the 3rd passage in RPMI at 10% FBS and then treated with CTLA4-Ig for 24 and 48 hours. Fibrocytes were characterized as CD45⁺CXCR4⁺COL1⁺ cells and the expression of CD86 and HLA-DR was also evaluated. The gene expression of aSMA, COL1, CXCR4, TGFβ1 and CD86 was investigated by quantitative real-time polymerase chain reaction in cultured fibrocytes and skin fibroblasts. In cultured skin fibroblasts, COL1 and fibronectin synthesis was evaluated by Western blotting.

Results: Treatment with CTLA4-Ig for 3 hours significantly downregulated aSMA and COL1 gene expression in cultured SSc fibrocytes at T8 (p<0.01, p<0.05 vs. untreated fibrocytes), whereas no modulatory effect was observed on the TGFβ1 and CXCR4 gene expression. In cultured SSc skin fibroblasts, CTLA4-Ig 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 CTLA4-Ig treatment than the skin fibroblasts isolated from the same SSc patient.

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Disclosure of Interests: Stefano Soldano: None declared, Paola Montagna: None declared, Sabrina Paolino: None declared, Elisa Alessandri: None declared, Carmen Pizzorni: None declared, Greta Pacini: None declared, Federica Goegan: None declared, Alberto Sulli Grant/research support from: Laboratori Baldacci, Carlotta Schenone: None declared, Vanessa Smith Grant/research support from: The affiliated company received grants from Research Foundation - Flanders (FWO), Belgian Fund for Scientific Research in Rheumatic diseases (FWRO), Boehringer Ingelheim Pharma GmbH & Co and Janssen-Cilag NV, Consultant of: Boehringer-Ingelheim Pharma GmbH & Co, Speakers bureau: Actelion Pharmaceuticals Ltd, Boehringer-Ingelheim Pharma GmbH & Co and UCB Biopharma Sprl, Maurizio Cutolo Grant/research support from: Bristol-Myers Squibb, Actelion, Celgene, Consultant of: Bristol-Myers Squibb, Speakers bureau: Sigma-Alpha

DOI: 10.1136/annrheumdis-2020-eular.4626

SAT0302

INNATE LYMPHOID CELLS INDUCE A FIBROTIC PHENOTYPE OF FIBROBLASTS

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Background: Fibrotic diseases are characterized by excessive extracellular matrix production as a result of immune-mediated permanent fibroblast activation. Innate 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 remodeling and fibroblast activation.

Methods: Skin biopsies of patients with systemic sclerosis (SSc) or scleroderma chronic graft versus host disease (scGvHD) as well as lung biopsies of patients with idiopathic pulmonary fibrosis (IPF) were analyzed by immunofluorescence (IF) staining. Single cell RNA-sequencing (scRNA-seq) was performed on ILCs 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 fibrotic activation. Quantitative real-time-PCR, western blot, IF staining and ELISA were used as readouts.

Results: Two different subtypes of ILC2s were found in skin of SSc and scGvHD patients as well as in lungs of IPF patients with one subpopulation 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 profibrotic ILC2s directly interacted with fibroblasts in a cell contact dependent manner. Semaphorin 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 emphasized the secretion of pro-fibrotic cytokines.

Conclusion: We identified a bidirectional interaction between ILCs and fibroblasts 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.

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Disclosure of Interests: Stefanie Weber: None declared, Charles Gwellem Anchang: None declared, Simon Rauber: None declared, Markus Luber: None declared, Maria Gabriella Raimondo Grant/research support from: Celgene, Partner Fellowship, Yuko Ariza Employee of: Ono Pharmaceutical Co., Ltd., Aleix Rius Rigau: None declared, Alexander Kreuter: None declared, Georg Schett Speakers bureau: AbbVie, BMS, Celgene, Janssen, Eli Lilly, Novartis, Roche and UCB, Jörg Distler Grant/research support from: Boehringer Ingelheim, Consultant of: Boehringer Ingelheim, Paid instructor for: Boehringer Ingelheim, Speakers bureau: Boehringer Ingelheim, Andreas Ramming Grant/research support from: Pfizer, Novartis, Consultant of: Boehringer Ingelheim, Novartis, Gilead, Pfizer, Speakers bureau: Boehringer Ingelheim, Roche, Janssen

DOI: 10.1136/annrheumdis-2020-eular.5804