– Rheumatology and Immunology, Erlangen, Germany; ²Ono Pharmaceutical Co., Ltd., Research Center of Speciality, Research Department, Osaka, Japan; ³University Witten-Herdecke, HELIOS St. Elisabeth Klinik Oberhausen, Oberhausen, Germany

Background: Systemic sclerosis (SSc) is a chronic autoimmune disease characterized by microangiopathy and fibrosis. In physiological wound healing, fibroblasts are transiently activated for tissue repair. In contrast, fibroblasts are persistently activated during fibrosis and thus resulted in progressive matrix deposition and tissue remodeling. However, the pathogenesis of the fibrotic process is not fully understood.

Objectives: We aimed to identify molecules that play a role in chronically activated fibroblasts.

Methods: To identify molecules specifically upregulated in human fibrotic fibroblasts, RNA-sequencing was performed. Identified adaptor proteins were further validated in skin biopsy samples of patients with limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc), and evaluated correlation between expression levels and clinical parameters. Respective overexpression and siRNA knockdown were further addressed *in vitro*. Functional effects were assessed by qPCR, hydroxyproline, proliferation and migration assays. Mouse models of systemic sclerosis were used to functionally validate adaptor proteins *in vivo*.

Results: We identified adaptor proteins as significantly upregulated molecules in chronically active fibroblasts of skin biopsy samples from SSc patients compared to fibroblasts from healthy controls. Expression levels were correlated with the modified Rodnan skin score in the skin of SSc patients. We observed higher expression levels also in the mouse model of topoisomerase I induced skin fibrosis. This result was also observed in bleomycin induced lung fibrosis model suggesting important functions of adaptor proteins during fibrotic tissue remodeling across different organs. Fibroblast-specific knockout resulted into significantly attenuated bleomycin-induced fibrosis. Upon bleomycin challenge, hydroxyproline content was diminished in mice with genetic deficiency of adaptor proteins. In addition, COL1A1, COL1A2 and Lum mRNAs and also the number of myofibroblasts were significantly lower in knockout mice compared to wild type mice. *In vitro*, knockdown of adaptor proteins resulted into a significant alteration of the migratory potential of fibroblasts.

Conclusion: Our results demonstrate that adaptor proteins play an essential role in the pathogenesis of systemic sclerosis. Understanding the molecular mechanism of adaptor proteins may lead to a novel therapeutic intervention in human SSc and related disorders.

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AB0151

PRELIMINARY RESULTS SHOW AN INCREASED EXPRESSION OF COINHIBITORY RECEPTORS IN SYSTEMIC SCLEROSIS

M. Aspari¹, S. R. Greisen^{1,2}, M. Hvid^{1,3}, B. Deleuran^{1,2}, D. Abraham⁴ on behalf of Bent Deleuran's Group David Abraham's Group. ¹Aarhus University, Biomedicine, Aarhus, Denmark; ²Aarhus University, Rheumatology, Aarhus, Denmark; ³Aarhus University, Department of Clinical Medicine, Aarhus, Denmark; ⁴University College London, Department of Cell and Molecular Biology, London, United Kingdom

Background: Recent studies suggest dysregulation in T cell activation in systemic sclerosis (SSc). Co-inhibitory-receptors (Co-IRs) such as TIM-3, PD-1 and LAG-3 play a crucial role in controlling excessive T cell activation and in maintaining immune homeostasis. Engagement of these receptors by their ligand's limits cytokine production in response to TCR or activating NK receptor stimulation and hence limit tissue damage from excessive immune activation. However, chronically increased expression of multiple Co-IRs is a hallmark of immune exhaustion. We evaluate the role of these soluble Co-IRs in diffuse SSc (dcSSc).

Objectives: Establish the role of CoIR and their ligands in diffuse systemic sclerosis.

Understand how immune regulatory mechanisms influence the development of fibrosis.

Provide a better understanding of the disease and fibrosis in general.

Methods: PBMCs (Peripheral blood mononuclear cells) and dermal fibroblasts from SSc patients were isolated and investigated for markers of T cell inhibition.

These cells were analysed using flow cytometry in a 10 colour panel. Cells were stained for PD1, TIM3, TIGIT, LAG3, CD3, CD8, CD4 and CD19 along with a Live/dead marker. Co-cultures of fibroblasts and PBMCs will be setup, and treated with various drugs that act on the Co-IRs.

Results: The proportion of CD4+ T cells expressing PD1 were markedly increased in SSc patients compared to healthy volunteers and Rheumatoid Arthritis patients.

There was increased expression of both TIGIT and TIM3 in the CD4+ T cells. (Figure 1)

Similarly, the co-expression of these receptors on the CD4+ T cell population was elevated compared to healthy volunteers. (figure 2)

Conclusion: Soluble co-inhibitors are differentially expressed in early dcSSc compared to healthy volunteers and other autoimmune diseases. Our preliminary data indicates that these co inhibitors could play an important role in unravelling the pathogenesis of systemic sclerosis. Inhibition or activation of these receptors through different treatment modalities can be utilized as a novel patient centric treatment strategy.

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Figure 1 CD3+CD4+ T cells Co inhibitor Receptor Expression

Figure 2 CD3+CD4+ T cells showing Co-expression of Co inhibitor Receptors

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AB0152

LENABASUM, A CB2 AGONIST, INHIBITS INFLAMMASOME ACTIVATION

S. Chintalacheruvu¹, P. Zang¹, B. White¹, S. Atamas¹. ¹Corbus Pharmaceuticals, Inc., Norwood, United States of America

Background: Upregulation of the innate immune response via the activity of Toll-like receptors and the NLRP3 inflammasome have been suggested as initiating events that can drive fibrosis in systemic sclerosis (SSc) (Pharmacol Ther. 2018;192:163). Lenabasum, a cannabinoid receptor type 2 agonist, is known to activate the resolution phase of acute human innate immune responses triggered through Toll-like receptor activation, favoring production of pro-resolving lipid mediators, reducing inflammatory infiltrates, and increasing bacterial clearance (Clin Pharmacol Ther. 2018;104:675). Given the potential importance of inflammasome activation in the pathogenesis of SSc, the question remained whether lenabasum inhibits inflammasome activation.

Objectives: Assess effects of lenabasum on IL-1 β and IL-18 production induced by inflammasome activation.

Methods: Primary human macrophages were derived from monocytes, stimulated with LPS and ATP to active inflammasomes and cultured with lenabasum. Levels of IL-1 β and IL-18 were measured in cell supernatants by ELISA. Separately, human PBMC were activated with 0.1 μ g/ml LPS $\pm$ 10 μ M lenabasum for 24 hours, and effects of lenabasum on the levels of IL-1 β and other pro-inflammatory cytokines were measured.

Results: Lenabasum significantly inhibited IL-1 β and IL-18 secretion by monocyte-derived macrophages, with IC₅₀ = 66.73 $\pm$ 3.92 nM and 349.23 $\pm$ 21.27 nM, respectively. A control inflammasome activation inhibitor, MCC950, which showed IC₅₀ = 18.33 $\pm$ 1.22 nM for IL-1 β inhibition and IC₅₀ = 21.43 $\pm$ 0.81 nM for IL-18 inhibition.

Conclusion: Lenabasum inhibits inflammasome activation, which could contribute to potential therapeutic efficacy in SSc and other autoimmune diseases.

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Figure

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AB0153

ADIPOSE-DERIVED STROMAL/STEM CELLS FROM SYSTEMIC SCLEROSIS PATIENTS SUCCESSFULLY EXERT A PARACRINE ANTI-FIBROTIC ACTIVITY AND INDUCE A PRO-ANGIOGENIC PHENOTYPE OF SCLERODERMA FIBROBLASTS IN VITRO

N. Del Papa¹, M. Lorini², V. Carbonelli², A. Minniti¹, F. Pignataro¹, W. Maglione¹, G. Di Luca³, N. Montano², R. Caporali¹. ¹ASST Gaetano Pini-CTO, Dept of Rheumatology, Milan, Italy; ²Ca' Granda IRCCS Foundation, Ospedale Maggiore Policlinico, Department of Clinical Sciences and Community Health, Milan, Italy; ³ASST Gaetano Pini-CTO, U.O.S Vascular Surgery, Milan, Italy

Background: Adipose-derived stromal/stem cells (ADSCs) are multipotential non-hematopoietic progenitor cells with anti-inflammatory, immunomodulatory and regenerative effects. They have the advantage of accessibility and potent pro-angiogenic effects when compared with other stem cells, such as bone-marrow derived stem cells. Recent studies have shown that autologous fat grafting may be effective in the treatment of fibrotic and vascular complications in systemic sclerosis (SSc), despite a pro-fibrotic signature.

Objectives: Aim of the study was to better characterize the proliferative and secretive profile of ADSCs in normoxic and hypoxic conditions, and to evaluate the mechanisms by which ADSCs exert these observed clinical effects.

Methods: Adipose tissue samples were obtained by liposuction from 12 SSc patients and 10 healthy donors (HD). ADSCs were purified according to their adherence to the plastic and characterized to express specific MSC surface antigens by flow cytometry analysis. Proliferation of ADSCs from SSc patients and normal controls was evaluated in normoxic and hypoxic conditions. Fibroblasts and ADSCs derived from SSc patients were co-cultured in direct and indirect culture systems and compared to HD. Fibroblasts proliferation, mRNA expression and protein secretion of VEGF and known fibrotic mediators including TGF β -1, TGFR, CTGF, Collagen type I (Col I) were analyzed in the same conditions.

Results: Normoxic and hypoxic culture conditions did not modify the proliferation rate of both normal and SSc ADSCs. Hypoxia significantly increased mRNA expression of VEGF by HD and SSc ADSCs but had no effect on the mRNA expression of pro-fibrotic mediators, ie TGF β and TGFR. Normal and SSc fibroblast proliferation was significantly reduced in both co-culture systems ($p < 0.001$) and by treatment with normoxic and hypoxic conditioned medium (CM) ($p=0.001$ and $p=0.002$). In the same conditions, mRNA expression and protein secretion of TGF- β 1, CTGF and Col I were significantly reduced ($p = 0.003$, $p = 0.02$, $p=0.04$). These results were confirmed when normal and SSc fibroblasts were cultured in the presence of ADSCs normoxic and hypoxic CM ($p=0.02$ and $p=0.01$). Furthermore, a significant increase in mRNA expression and production of VEGF was observed in SSc fibroblasts cultured in the presence of normoxic and hypoxic CM ($p = 0.002$ and $p < 0.0001$, respectively).

Conclusion: We found that treatment with the medium from normoxic and hypoxic preconditioned ADSCs has an anti-fibrotic effect through both the inhibition of fibroblast proliferation and key mediators of fibrosis. The increased expression of VEGF by SSc fibroblasts in the presence of normoxic and, even more, hypoxic ADSCs CM, suggests that ADSCs can induce a paracrine pro-angiogenic phenotype, even in fibroblasts with a pro-fibrotic signature. Altogether these data show that ADSCs may exert their anti-fibrotic and pro-angiogenic effects on SSc fibroblasts by the secretion of paracrine factors, partly explaining the mechanisms underlining beneficial clinical results of fat graft in SSc patients.

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AB0154

MERTK AND THE RESOLUTION OF INFLAMMATION IN IGG4-RELATED DISEASE

E. Della Torre¹, L. Rovati¹, A. Monno¹, T. Maehara², N. Kaneko², M. Lanzillotta¹, F. Pedica³, C. Doglioni³, S. Pillai⁴, A. Manfredi⁵. ¹San Raffaele Hospital, Unit of Immunology Rheumatology Allergy and Rare Diseases (UnIRAR), Milan, Italy; ²Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan; ³San Raffaele Hospital, Pathology Unit, Milan, Italy; ⁴Ragon Institute of MGH, MIT, Harvard, Boston, United States of America; ⁵San Raffaele Hospital, Autoimmunity and Vascular Inflammation Lab, Milan, Italy

Background: IgG4-Related Disease (IgG4-RD) is characterized by fibrotic lesions, serum IgG4 elevation, and prompt response to glucocorticoids. B and T lymphocytes are considered the initiators of tissue inflammation in IgG4-RD, but the prominent stromal reaction observed at disease sites suggest that a dysregulation of processes involved in the resolution of inflammation could be pathologically relevant as well. Mer receptor tyrosine kinase (MerTK) and its ligands protein S (Pros1) have a pivotal role in the resolution of inflammation through the activation of a well-characterized signaling pathway that ultimately dampens the immune response and promotes the recovery of tissue function. MerTK and the processes involved in the resolution of inflammation have never been addressed in IgG4-RD.