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

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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 secretory 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

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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.

Objectives: To investigate MerTK involvement in the pathogenesis of IgG4-RD by evaluating (a) the expression of MerTK and of its endogenous ligands in IgG4-RD tissues; (b) the presence of circulating precursors of MerTK+ cells infiltrating IgG4-RD lesions in the peripheral blood of IgG4-RD patients; (c) the effects of immunosuppressive therapies on MerTK expression in IgG4-RD tissues.

Methods: Three distinct cohorts of IgG4-RD patients were included in this study. 8 active patients were used for immunohistochemistry studies for MerTK expression. 16 IgG4-RD and 14 Sjögren syndrome patients, together with 6 control tonsils, were used for multicolor immunofluorescence studies and TissueQuest software quantification of the expression of MerTK, CD68, CD163, Pros1, Gas6, CD4, SLAMF7, CD19, IgG4, cleaved caspase-3. 10 untreated IgG4-RD patients were used to evaluate MerTK expression in circulating monocytes subsets and fibrocytes by flow cytometry.

Results: MerTK was highly expressed in IgG4-RD affected organs. MerTK+ cells accounted on average for 16% (range 5-35%) of all cells in the tissue, and the majority of them expressed CD68, reflecting a monocyte-macrophage origin. 33.5 % (interquartile range (IQR) 26-41%) of MerTK+ cells co-expressed CD68 and CD163, while 30.5% (IQR 19-41.5%) expressed CD68 but not CD163. CD68+MerTK+ cells displayed two main morphological appearances, compatible with those of macrophages and of myofibroblasts. In addition, MerTK+ cell number was significantly increased in salivary glands from IgG4-RD patients compared to Sjögren syndrome ($p < 0.0001$). Circulating precursors of CD68+MerTK+ cells infiltrating IgG4-RD lesions were identified by flow cytometry in the peripheral blood of patients with active IgG4-RD as MerTK+ populations of intermediate monocytes, nonclassical monocytes and collagen expressing fibrocytes. MerTK ligand Pros1 was exposed on 52% (IQR 42-57%) of infiltrating B lymphocytes, 74% (IQR 54-89%) of infiltrating T lymphocytes, and, likely, on apoptotic cells that were detected in IgG4-RD tissues. CD68+MerTK+ cells were found in physical contact with Pros1+ cells in IgG4-RD lesions and their number decreased by 56% after successful treatment with rituximab.

Conclusion: MerTK is abundant in IgG4-RD affected organs and is preferentially expressed on CD68+ macrophages and myofibroblasts that infiltrate IgG4-RD lesions. MerTK+ cells might interact with apoptotic cells and Pros1 expressing T and B lymphocytes in IgG4-RD tissues, leading to the persistent activation of processes involved in the resolution of inflammation and promoting the development of tissue fibrosis.

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AB0155

THE ULTRASTRUCTURAL FEATURES OF INTERSTITIAL LUNG TISSUE INVOLVEMENT DUE TO SYSTEMIC SCLEROSIS: ANIMAL EXPERIMENTAL STUDY

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Background: Systemic sclerosis (SSc) - is an autoimmune connective tissue disease, which crucial role is played by inflammation and fibrosis development. This pathology is characterized by multiple symptoms that occur due to alterations of the vascular wall, limited or widespread fibrosis of the dermis and visceral organs, dysregulation of cellular and humoral immunity, followed by the formation of autoantibodies specific to the structures of the organism. Although external manifestations of SSc certainly lead to a decrease in the functional activity and patient's quality of life up to disability, the real danger is a violation of the functions of the internal organs and systems.

Objectives: The aim of our study was to determine changes in the morphological structure of lung interstitial tissue at the subcellular level of the structural organization, which were achieved by modeling of this autoimmune pathology in laboratory animals.

Methods: The main concept of modeling process was based on the previously described method of SSc induction [1,2] however with some differences from original model. Into the study were involved 30 pubescent Wistar rats (220-240g) who underwent a three times a week subcutaneous administration of 0.5ml of 5% sodium hypochlorite (NaClO) solution with active chlorine concentration of 190 g/dm³ for 6 weeks in a row; control group (20 rats) that received injections with an equal volume of sterile saline solution. After

8 weeks from the beginning of the experiment animals were sacrificed under thiopental anesthesia, lung tissue specimens were obtained, fixed alternately in 2.5% glutaraldehyde and in 1% solution of osmium tetroxide. After dehydration, the material was embedded in Epon-Araldite. Sections were obtained on an ultramicrotome (Tesla BS-490) magnification x9600 was performed on high-resolution microscope (PEM-125K).

Results: The ultrastructural study of lung specimens revealed the fibroblasts with high amounts of collagen fibers in the interstitial tissue of the alveoli wall. Fibroblast nuclei were characterized by irregular shape and numerous invaginations of the nuclear envelope and marginal aggregation of chromatin granules. Fig. 1 depicts the fragment of the alveolar wall of the lungs of white Wistar rats in 8 weeks after the start of the experiment. On the Fig. 1 could be distinguished the lumen of the alveoli (1), fibroblast (2) with increased quantity of collagen fibers (3) and a peripheral part of alveolocyte I type (4).

Figure 1.

Conclusion: The current findings confirm the efficacy of NaClO chemical model of reproduction of interstitial lung tissue involvement within SSc pathogenesis; this model could contribute to the further investigation of peculiarities of autoimmune origin interstitial lung disease (ILD).

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AB0156

NMR-BASED MUSCLE METABOLOMICS IN INFLAMMATORY MYOSITIS- UNDERSTANDING CHANGES IN SERUM AS A REFLECTION OF THE MUSCLE

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Background: We have previously found promise in NMR as a tool to distinguish sera of active from inactive inflammatory myositis (IIM). To understand the changes previously found in sera and urine we studied muscle tissue of patients with myositis.

Objectives: To identify differences in metabolome on inflamed muscle tissues of patients with active myositis from that of healthy controls and infectious polymyositis.

Methods: Muscle (n=17) from patients classifiable as myositis by the ACR-EULAR criteria [34 years (23.5 - 50.5 IQR), M/F 1:3] were compared with healthy controls [n=11, age= 44 (35-50) years, M/F-1:1]. Two disease controls with infectious polymyositis were also compared. Findings were applied to muscle biopsy tissues of two patients with established myositis and superadded infections (HBV, Histoplasmosis) to assess discriminatory potential.