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, Pro51,Ga65; 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.

Disclosure of Interests: None declared

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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 x600 was performed on high-resolution microscope (PEM-125K).

Results: The ultrastructural study of lung specimens reviled 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 type (4).

Figure 1.

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

References:

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AB0156

NMR-BASED MUSCLE METABOLICOMICS 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.
Conclusions: Succinate and elevated citrate in both patients with IIM with superadded infectious polymyositis also exhibited low levels, suggesting metabolomics could possibly be useful to differentiate the two. Muscle biopsies of infectious polymyositis suggested differences in spectra from IIM (Fig. 1A). Of the various discriminatory metabolites (Fig. 1B), succinate had the highest discriminatory potential (AUC 0.8, P<0.01) followed by citrate, glycine, glycerol, glucose, creatine and lactate. Both glucose and creatine were decreased in IIM (Fig. 1D,E) and this was uniform across all types of IIM. However, glycine levels differed across different myositis subsets supporting the fact that they might differ in pathogenesis. (Fig. 1E) Amongst various serum biomarkers of muscle disease and damage, serum Aspartate Transaminase correlated positively with glutamate (r=0.6, P<0.01), and serum creatine correlated negatively with glycine (r=-0.8, P<0.04).

Biopsies of infectious polymyositis suggested difference in spectra from IIM (Fig. 2A). Trends were observed towards lower succinate and higher citrate levels suggesting metabolomics could possibly be useful to differentiate the two. Muscle of both patients with IIM with superadded infectious polymyositis also exhibited low succinate and elevated citrate. Conclusion: Muscle metabolomics of active myositis is distinctive. Amino acids and creatine are lower in diseases muscle suggesting active breakdown and loss, in turn explaining previous findings of low levels in serum in active disease. Certain metabolite composition differ in different types of myositis supporting different pathogenesis. Infectious polymyositis might exhibit different metabolome from IIM with potential as a biomarker though this needs to be confirmed in larger numbers.

Results: Metabolomics profiles in IIM were distinct from healthy controls (Fig. 1A). Of the various discriminatory metabolites (Fig. 1B), succinate had the highest discriminatory potential (AUC 0.8, P<0.01) followed by citrate, glycine, glycerol, glucose, creatine and lactate. Both glucose and creatine were decreased in IIM (Fig. 1D,E) and this was uniform across all types of IIM. However, glycine levels differed across different myositis subsets supporting the fact that they might differ in pathogenesis. (Fig. 1E) Amongst various serum biomarkers of muscle disease and damage, serum Aspartate Transaminase correlated positively with glutamate (r=0.6, P<0.01), and serum creatine correlated negatively with glycine (r=-0.8, P<0.04).

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