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THU0351 LOW ERA EXPRESSION MIGHT BE IMPLICATED IN THE DEVELOPMENT OF PULMONARY HYPERTENSION IN FEMALE MICE LACKING PSGL-1

Rafael González-Tajuelo¹, María De la Fuente-Fernández¹, Daniel Morales-Cano², Antonio Muñoz-Callejas¹, Juan Manuel Serrador³, Bianca Barreira⁴, Javier Silván¹, Carlos Gamallo⁵, Esther Vicente⁶, Santos Castañeda⁷, Francisco Pérez-Vizcaino⁴, Angel Cogolludo⁴, Ana Urzainqui¹. ¹Instituto de Investigación Sanitaria-Princesa, FIB-Hospital de la Princesa, Inmunología, Madrid, Spain; ²University Complutense of Madrid, Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Pharmacology, Madrid, Spain; ³Centro de Biología Molecular "Severo Ochoa" (CBMSO), CSIC-UAM, Cellular Biology and Immunology, Madrid, Spain; ⁴University Complutense of Madrid, Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Pharmacology, Madrid, Spain; ⁵Instituto de Investigación Sanitaria-Princesa, FIB-Hospital de la Princesa, Inmunología, Madrid, Spain; ⁶Instituto de Investigación Sanitaria-Princesa, FIB-Hospital de la Princesa, Rheumatology, Madrid, Spain; ⁷Instituto de Investigación Sanitaria-Princesa, FIB-Hospital de la Princesa, Rheumatology, Madrid, Spain

Background: Pulmonary arterial hypertension (PAH) is a rare disease with unknown etiopathogenesis and no curative treatment [1]. PAH is one of the major complications of connective tissue diseases, and 7-15% of patients with systemic sclerosis (SSc) develop PAH [2]. Mice deficient for the leukocytic receptor P-selectin glycoprotein ligand-1 (PSGL-1^{-/-}) spontaneously develop a SSc-like autoimmune syndrome with ageing [3].

Objectives: To check whether PSGL-1^{-/-} mice may develop PAH and the molecular mechanisms that might be implicated in the initiation and establishment of the disease.

Methods: Doppler pulse echocardiography was used to evaluate pulmonary artery flow acceleration time/ejection time (PAAT/ET) ratio in WT and PSGL-1^{-/-} C57BL/6 mice. Isolated pulmonary artery rings were incubated with acetylcholine and responses were registered with a wire myograph coupled to an isometric force transducer. Expression levels of the NO-sensing probe DAR-4M AM by fluorescence microscopy and flow cytometry. Angiotensin II lung concentration was quantified by ELISA. eNOS, p-ENOS, AT1R and AT2R expression was evaluated by western blot. In all cases, data are expressed as the mean±SD.

Results: Aged PSGL-1^{-/-} females showed reduced flow PAAT/ET ratio indicating PAH. Moreover, pulmonary arterial rings from aged PSGL-1^{-/-} females presented ROS-independent reduced vasodilation response to acetylcholine. Importantly, eNOS phosphorylation was impaired and NO production by lung EC was reduced in aged PSGL-1^{-/-} females. Vascular remodeling and reduced expression of AT2R were observed in lungs of PSGL-1^{-/-} females from a younger age. With ageing, the levels of angiotensin II and the percentages of IFN γ -producing interstitial macrophages, T and B lymphocytes were increased in PSGL-1^{-/-} females. The differences in the gender-biased genotype could be explained by the reduced expression of ER α in the lungs of aged PSGL-1^{-/-} females while WT and PSGL-1^{-/-} males showed similar expression.

Conclusion: PSGL-1 deficiency leads to pulmonary hypertension in >18-months-old female mice, involving various mechanisms:

1. Lung vessel wall remodeling and reduced AT2R expression.
2. Reduced eNOS phosphorylation and reduced NO production with the subsequent specific lung endothelial dysfunction in PSGL-1^{-/-} females. Importantly, ROS production is not increased nor help to NO reduction.
3. Increased pulmonary AngII levels with ageing in PSGL-1^{-/-} females.
4. Increased Th1 polarization, reduced Treg population and increased IFN- γ production by interstitial macrophages.

Impaired ageing up-regulation of ER α expression in the lungs of PSGL-1^{-/-} females.

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THU0352 THE ROLE OF PRURITOGENIC MEDIATORS IN DERMATOMYOSITIS RELATED ITCH

Anett Vincze¹, Erika Herczeg-Lisztes², Katalin Szabó¹, Melinda Nagy-Vincze¹, Tamás Biró³, Katalin Dankó¹, István Balázs Tóth², Zoltán Griger¹. ¹Faculty of Medicine, University of Debrecen, Division of Clinical Immunology, Department of Internal Medicine, Debrecen, Hungary; ²Faculty of Medicine, University of Debrecen, Department of Physiology, Debrecen, Hungary; ³Faculty of Medicine, University of Debrecen, Department of Immunology, Debrecen, Hungary

Background: Pruritus is a common symptom in systemic autoimmune diseases like dermatomyositis (DM). Recent researches have indicated that interleukin-31 (IL-31), IL-33, IL-6, or inflammatory cytokines, such as tumor necrosis factor (TNF α), peroxisome proliferator-activated receptor γ (PPAR γ) and ion channels belonging to the transient receptor potential (TRP) family are involved in pruriception.

Objectives: We examined targeted gene expression analysis of lesional versus non-lesional skin samples of patients affected with active DM. We looked for correlations between the examined pruriceptive signaling molecules, disease activity and itching sensation of DM patients.

Methods: Gene expression of TNF α , PPAR γ , IL-33, IL-6 and TRPV channels in lesional DM skin was evaluated by RT-qPCR and was compared with non-lesional DM skin samples. Pruritus and disease activity of DM was evaluated by the 5-d itch scale and Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI), respectively. Statistical analysis was performed with IBM SPSS 20.0 software.

Results: Skin samples of 17 active DM patients were analyzed. We could show, that itching index in DM was positively correlated with CDASI score with a correlation coefficient of 0.82 ($p < 0.001$). TNF α gene expression was significantly higher (34.87%) in lesional DM skin than non-lesional DM skin ($p = 0.03$). The normalized TNF α mRNA expression was positively correlated with itch scale ($R = 0.605$, $p = 0.022$) and its level was significantly higher in skin samples of patients with severe itch (itching score: 15-20) versus mild itch (itching score: 5-10) (2.02 ± 0.38 vs. 1.12 ± 0.20 ; $p < 0.01$). The level of PPAR γ was decreased in lesional DM skin, but this was statistically not significant. The mRNA expression of normalized PPAR γ was negatively correlated with itch scale ($R = -0.618$, $p = 0.019$), and its level was significantly lower in skin samples of patients with severe itch versus mild itch (0.28 ± 0.36 vs. 1.53 ± 0.98 ; $p = 0.038$). Lesional IL-6 mRNA levels were associated with CDASI activity score ($R = 0.619$, $p = 0.018$). The mRNA levels of TRPV1-4 channels were not associated with 5-D itch score, but normalized TRPV1 and TRPV4 mRNA expressions were positively correlated with CDASI damage score ($R = 0.699$, $p = 0.008$; $R = 0.789$, $p = 0.001$). Interestingly, itching sensation of DM patients was not correlated with IL-33 mRNA levels measured in skin samples.

Conclusion: Our results argue for that higher cutaneous disease activity generate pruritus. TNF α and PPAR γ might play a determining, but opposite role in DM-associated itch. Furthermore IL-6, TRPV1 and TRPV4 channels might participate in pathomechanism of cutaneous manifestation of the disease.

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THU0353 AN ORALLY AVAILABLE HIGHLY SELECTIVE 5-HYDROXYTRYPTAMINE 2B (5-HT_{2B}) RECEPTOR ANTAGONIST AMELIORATING PULMONARY AND DERMAL FIBROSIS IN PRECLINICAL MODELS OF SYSTEMIC SCLEROSIS

Christina Wenglén, Helena Arozenius, Lars Pettersson, Gunilla Ekstrom. AnaMar AB, Medicin Village AB, Lund, Sweden

Background: Serotonin or 5-hydroxytryptamine (5-HT) is well known as a stimulator of tissue fibrosis and a significant role of peripheral 5-HT_{2B} receptors in fibrosis has been suggested with the receptor being upregulated in fibrotic tissues. In addition, agonism of the 5-HT_{2B} receptor has been implicated in human tissue fibrosis caused by drugs known to activate the receptor. Pharmacologic inhibition of 5-HT_{2B} receptor signalling consequently represents a promising treatment strategy for fibrotic disorders including systemic sclerosis. 5-HT is released from platelets activated upon vascular damage, one of the first pathological events in systemic sclerosis. The local 5-HT concentration is increased and leads to activation of 5-HT_{2B} receptors on e.g. fibroblasts. The pro-fibrotic effects of 5-HT and the 5-HT_{2B} receptor are believed to be mediated through activation of the TGF- β /Smad signaling pathway.

Objectives: The objective of the present study was to evaluate a novel highly selective orally available 5-HT_{2B} receptor antagonist, AM1476, for its ability to reduce pulmonary and dermal fibrosis in the sclerodermatous

chronic graft-versus-host disease model and dermal fibrosis in the tight-skin-1 model of systemic sclerosis. Effects on the TGF- β /Smad signaling pathway were investigated *in vivo*. Exposure of AM1476 was measured to ensure proper target engagement.

Methods: The murine sclerodermatous chronic graft-versus-host disease (cGvHD) model was used to evaluate anti-fibrotic effects after therapeutic dosing of the 5-HT_{2B} receptor antagonist, AM1476. The compound was orally administered at 1, 10 and 30 mg/kg b.i.d. from day 21, several days after the first clinical signs of cGvHD, to day 49. Dermal thickness, myofibroblast counts and collagen production were used to evaluate dermal fibrosis. Effects on pulmonary fibrosis were measured using hydroxyproline content, Sirius Red staining and Ashcroft score. Plasma was collected for PK analysis at different timepoints in each treatment group using sparse sampling, on the last day of the experiment. The tight-skin-1 model was used to evaluate anti-fibrotic effects after therapeutic treatment. AM1476 was orally administered at 10 mg/kg, b.i.d. from week 5 to week 10. Hypodermal thickening, myofibroblast counts and hydroxyproline content in skin biopsies were evaluated at the end of the treatment period. The number of phosphorylated Smad3 (pSmad3) positive cells was used to evaluate inhibition of TGF- β signaling.

Results: The 5-HT_{2B} receptor antagonist AM1476, significantly reduced all measured dermal and pulmonary fibrosis readouts in the cGvHD model using an oral therapeutic treatment approach. Pharmacokinetic analysis of plasma samples supported 5-HT_{2B} receptor engagement. Therapeutic treatment of dermal fibrosis in the tight-skin model effectively and significantly reduced hypodermal thickening, number of myofibroblast and hydroxyproline content. The number of pSmad3 positive cells was significantly reduced in skin samples isolated from treated animals.

Conclusion: Inhibition of 5-HT_{2B} receptor activity resulted in pronounced anti-fibrotic effects in pulmonary and dermal fibrotic tissues. AM1476 was well tolerated without obvious signs of toxicity. Effects on pSmad3, reflecting TGF- β -inhibition, will be evaluated as a potential biomarker in upcoming clinical trials. The highly selective 5-HT_{2B} receptor antagonist AM1476 represents a promising drug candidate for treatment of fibrotic conditions and is currently in development for systemic sclerosis.

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THU0354

MACHINE LEARNING CLASSIFICATION OF SKIN GENE EXPRESSION IDENTIFIES A SUBSET OF SYSTEMIC SCLEROSIS PATIENTS MOST LIKELY TO SHOW CLINICAL IMPROVEMENT IN RESPONSE TO ABATACEPT

Jennifer Franks¹, Bhaven Mehta¹, Veronica Berrocal², Yue Wang¹, Tammara Wood¹, Christopher Denton³, Robert Lafyatis⁴, David Fox², Dinesh Khanna², Michael Whitfield¹. ¹Geisel School of Medicine at Dartmouth, Hanover, United States of America; ²University of Michigan, Ann Arbor, United States of America; ³Royal Free Hospital, London, United Kingdom; ⁴University of Pittsburgh, Pittsburgh, United States of America

Background: A prior pilot study of abatacept in SSc with molecular gene expression data in skin (1) found that four of five patients who improved on abatacept, as determined by change in mRSS, were in the inflammatory intrinsic gene expression subset. Improvement was accompanied by a decrease in gene expression for immune pathways, including the CD28 and CTLA4 receptors—the target of abatacept. Here we tested our *a priori* hypothesis that the inflammatory subset shows a significant decline in mRSS during abatacept therapy.

Objectives: ASSET (Abatacept Systemic Sclerosis Trial) is a Phase 2 study designed to assess the efficacy of subcutaneous abatacept to treat diffuse cutaneous systemic sclerosis. We performed RNA-sequencing (RNA-seq) analysis of skin biopsies and analyzed associations between intrinsic molecular subsets and clinical outcomes in the ASSET trial.

Methods: RNA-seq was performed on skin biopsies from 84 participants in the ASSET trial. A machine learning classifier was trained on independent cohorts and used to objectively classify patients into intrinsic gene expression subsets prior to study unblinding (2). Treatment differences in longitudinal outcomes were assessed using linear mixed effect models.

Results: 84 participants were assigned to intrinsic subset at baseline resulting in 33 inflammatory, 18 fibroproliferative, and 33 normal-like samples. Change in mRSS over 12 months was significantly different between the abatacept and placebo treatment arms for the inflammatory ($p < 0.001$) and normal-like ($p = 0.03$) subsets, but there was no difference in the fibroproliferative subset of patients ($p = 0.47$) (Figure 1). For FVC%

predicted, the fibroproliferative subset showed a numerical increase in FVC% in the abatacept arm ($p = 0.19$) while all other groups showed decreases in FVC%. All gene expression subsets showed decreases in HAQ-DI in the abatacept arm not observed in the placebo arm, with the most robust changes occurring in the inflammatory ($p = 0.09$) and normal-like ($p = 0.06$) subsets.

Conclusion: There was a marked divergence of the trajectory for mRSS change for the inflammatory subset and no apparent impact for the fibroproliferative subset. In contrast, only the fibroproliferative subset showed a clinically meaningful FVC change for abatacept. For the broader functional measure of HAQ-DI, there were similar impacts of abatacept on all three intrinsic skin gene expression subsets but is numerically greatest for the normal-like and fibroproliferative subsets. Together these data show for the first time in a placebo-controlled trial that intrinsic skin gene expression subsets may predict differential response to a targeted biological therapy, and also that this may reflect impact on different facets of SSc pathogenesis in skin or lung. This suggests that stratification of cases according to intrinsic gene expression subsets may maximize the number of informative SSc cases in clinical trials, and potentially improve future clinical practice.

Abstract THU0354 – Figure 1

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