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THU0355 IGURATIMOD MIGHT TREAT SCLERODERMA WITH INTERRUPTED EGR1/TGF- β LOOP

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized with multiple organ fibrosis. Previous studies showed transcription factor early growth response 1 (Egr1) overexpressed in the lesional skin of SSc patients, as well as Egr1 inducible genes¹. Egr1 forms a positive feedback loop with master pro-fibrotic cytokine TGF β and thus promotes fibrosis.

Objectives: To investigate the anti-fibrotic effect of a novel DMARD iguratimod in scleroderma models and patient skin grafts.

Methods: We used iguratimod to treat TGF β -stimulated human skin fibroblast, bleomycin induced mice, tight skin 1 (TSK-1) mice and SSc skin grafts. The bleomycin model contained pre-establish fibrosis and late onset treatment. The skin grafts came from three SSc patients and was planted into irradiated nude mice.

Results: Iguratimod down-regulated egr1 expression in human skin fibroblast, with decreased collagen production and α -SMA expression. Knocking down Egr1 in fibroblast could mimic these effects. Both oral and topical iguratimod could reduce dermal thickening and collagen deposition in bleomycin induced skin fibrosis. α -SMA (+) myofibroblast counts, as well as Egr1 (+) and/or TGF β (+) fibroblast counts in iguratimod treated groups were significantly less than the controls. Similarly, topical iguratimod ameliorated fibrosis with deduced dermal thickening in TSK-1 mice. Of note, 5-week iguratimod local injection remarkably reduced collagen content in skin grafts from three SSc patients. Staining of Egr1 and TGF β in skin tissue were inhibited after iguratimod treatment simultaneously.

Conclusion: We found the potential of iguratimod to treat SSc, which was characterized as an Egr1 inhibitor. Further clinical investigation is needed to establish its safety and efficacy.

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THU0356 STAPHYLOCOCCUS AUREUS REGULATES FIBROBLAST FUNCTIONS – IMPLICATIONS FOR TISSUE REPAIR IN CHRONIC DIGITAL ULCERS IN SYSTEMIC SCLEROSIS

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Background: Chronic digital ulcers (DU) are a major complication in systemic sclerosis (SSc). Non-healing wounds are characterized by persistent inflammation, defective re-epithelialization and impaired matrix remodeling,

and are often accompanied by bacterial colonization. In DU, the breach of the basement membrane exposes tissue and cell layers to commensal skin bacteria.

Objectives: To investigate whether interactions between commensal skin bacteria and dermal fibroblasts affect tissue repair mechanisms.

Methods: Dermal fibroblasts isolated from healthy controls (HC) and patients with diffuse cutaneous SSc (dcSSc) (n=3, each) were co-cultured with *Staphylococcus aureus* (SA) for 3 h at using a lower inoculum rather representing colonization (1×10^5 CFU/ml) and a higher inoculum reflecting infection (1×10^7 CFU/ml). Thereafter, fibroblasts were cultured with flucloxacillin-containing medium to kill extracellular and adherent bacteria. For mechanistic studies, a fibroblast cell line (BJ-5ta) was used. IL-6, IL-8, pro-collagen I α , and interferon (IFN)- β proteins in culture supernatants were measured by ELISA. Gene expression was assessed by quantitative PCR. Contractility was analyzed by collagen gel contraction assay. Wound closure assay was performed on a confluent cell layer with a uniform cell-free gap, generated by a cell culture insert. Expression of α -smooth muscle actin (α -SMA) was evaluated by Western blotting, cell proliferation with a colorimetric assay. Cell death was analyzed by live cell real time assay. Bacterial invasion was assessed by immunofluorescence staining using confocal microscopy and CFU recovery. Gene knockdown was performed using siRNA.

Results: Exposure to 1×10^7 CFU/ml of SA influenced fibroblast function. In brief, exposure to SA increased the secretion of IL-6 and IL-8 in dermal fibroblasts by 1571/455-fold (HC/dcSSc; p=0.08/<0.05) and by 325/1402-fold (HC/dcSSc; p<0.05 each) respectively. In addition we observed upregulation of the expression of MMP1 by 8.2/5.7-fold (HC/dcSSc; p<0.05 each) and MMP3 by 5.1/5.7-fold (HC/dcSSc; p=0.06/<0.05). There was a trend towards decreased secretion of pro-collagen I α . TGF- β -induced α -SMA expression was completely inhibited. Cell contraction was impaired by 22.0/31.6% (HC/dcSSc; p=0.08/<0.05), wound closure by 18.8/14.9% (HC/dcSSc; p<0.05 each), and proliferation by 30.5/44.3% (HC/dcSSc; p=0.24/<0.05). SA induced apoptosis and necrosis. SA invaded fibroblasts via endocytosis (25-50% of inoculated live SA). IFN- β secretion was induced 8.4/31.7-fold (HC/dcSSc; p<0.05 each). The genes of cytosolic dsDNA sensor molecules such as cyclic GMP-AMP synthase (cGAS) were upregulated 11.9/8.6-fold (HC/dcSSc; p<0.05 each). TLR9, an endosomal DNA sensor, was constitutively expressed. Knockdown of STING or MyD88, downstream mediators of cGAS and TLR9 respectively, reduced the induction of IFN- β (down to 68.2/66.2%; p=0.20/0.16), IL-6 (down to 57.2/55.2%; p<0.05 each), and IL-8 (down to 45.3/36.0%; p<0.05 each). Furthermore, inhibition of endocytosis with cytochalasin D decreased the expression of IFN- β (down to 24.6%; p<0.05), IL-6 (down to 34.9%; p<0.05), and IL-8 (down to 27.6%; p<0.05). In contrast, inhibition of endocytosis did not affect induction of apoptosis. This suggests that the invasion of fibroblasts by SA and the subsequent intracellular DNA sensing are crucial for the induction of these genes independently of apoptosis.

Conclusion: Invasion of dermal fibroblasts by SA with activation of the STING and TLR9-MyD88 pathways is a key element in the impairment of tissue repair responses.

THU0357 VENTRICULAR-ARTERIAL COUPLING AS A PREDICTOR OF CARDIOVASCULAR EVENTS IN SYSTEMIC SCLEROSIS

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Background: Standard Transthoracic Echocardiography (TTE) parameters have shown a low sensitivity in the detection of primary myocardial involvement (PMI) in systemic sclerosis (SSc). Arterial-ventricular coupling (VAC) is calculated by TTE as the ratio between arterial elastance (Ea) and ventricular end-systolic elastance (Ees), which reflects left ventricle stiffness. VAC is a central determinant of cardiovascular performance.

Objectives: We aimed to assess TTE-derived measures of cardiac mechanics and the prognostic role of VAC in SSc.

Methods: 75 patients affected by SSc without symptoms of cardiac involvement and 10 controls matched for sex and age were retrospectively evaluated. We considered the first TTE available after SSc diagnosis. Determinants of cardiac mechanics were calculated by TTE¹: end systolic elastance (Ees), arterial elastance (Ea), ventricular arterial coupling (VAC=Ees/Ea), end diastolic elastance (Eed). A value of VAC > 0.62 was considered indicative of altered VAC (i.e. uncoupling), whereas a lower value as normal (i.e. coupling). Finally, we considered the hospitalization for cardiovascular event during a mean follow-up of 10.8 $\pm$ 1.8 years.