AB0029 ANTI INFLAMMATORY EFFECT OF PDE5 INHIBITION: SCOPE FOR A NEW POTENTIAL INDICATION IN SSC ASSOCIATED MYOSITIS

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Background: Skeletal muscle damage can occur as clinical manifestation of Systemic Sclerosis (SSc) [1], and it is known to be associated to type I IFN pathway activation [2]. The type I IFN-induced chemokine CXCL10 is associated with a more severe SSc prognosis and skeletal muscle disease [3,4], and it has been reported to play a role in inflammatory myopathy (IM) and in diabetic cardiomyopathy (DCM) [5,6]. In DCM the phosphodiesterase type 5 inhibitor (PDE5i) sildenafil significantly decreased CXCL10 systemic and cellular release [7]. In SSc, sildenafil is used to treat pulmonary artery hypertension (PAH) and digital ulcers disease.
Objectives: To determine the serum levels of CXCL10 in SSc patients with or without muscle involvement and treated or not with sildenafil. To determine the role of sildenafil on IFNγ+TNFα-induced CXCL10 release in human skeletal muscle cells (Hfsmc).
Methods: Sera from 109 patients fulfilling ACR/EULAR 2013 classification criteria for SSc and 34 age and gender matched healthy controls (HC) were analysed by multiplexed, bead-based immunoassay. Hfsmc were cultured and analysed as previously described [5].
Results: CXCL10 serum level was significantly higher in SSc vs. HC (602.1±155.1 pg/ml vs 197.5±14.9 pg/ml, $P<0.001$) independently of diffuse or limited clinical subset ($p>0.05$); the presence of sildenafil in therapeutic regimen was associated with lower serum CXCL10 (455.2±211.8 pg/ml vs 633.1±183.02 pg/ml, $P<0.05$). CXCL10 was significantly higher in patients with increased Creatine Kinase (CK) (2703±2172 vs 454±82.51, $P<0.01$) and its concentration strongly correlated with the levels of CK (r=0.986, $P<0.001$) and with Medsger muscle Severity score (r=0.445, $P<0.001$). *In vitro*, sildenafil suppressed IFNγ+TNFα-induced CXCL10 release by Hfsmc in a dose dependent manner, down to 50% secretion at 1mM ($P<0.05$). The inhibition of CXCL10 secretion was associated with significant reduction in cytokine-induced STAT1, NFKB and JNK phosphorylation ($P<0.01$).
Conclusions: High CXCL10 level is associated with SSc independently from local or diffuse clinical subset and is lower in patients assuming sildenafil independently of other therapies. The strong correlation of CXCL10 and severity of muscle damage as assessed by serum CK and Medsger Muscle severity score, strongly indicate/confirm the involvement of IFN-I pathway activation during myositis in SSc. The direct inhibitory effect of PDE5 inhibitor Sildenafil on proinflammatory induced CXCL10 secretion warrant further research on the potential role of PDE5 inhibitors as disease modifying agents in SSc.
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AB0030 THE EFFECT OF SILDENAFIL AND ILOPROST ON CXCL10 LEVEL IN SYSTEMIC SCLEROSIS: IN VIVO AND IN VITRO COMPARISON

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Background: Th1 cell/cytokine repertoire contributes to systemic sclerosis (SSc) pathogenesis from early autoimmune/vascular stages while Th2 dominance prevails later, when (multi)organ fibrosis occurs [1]. The Th1-type chemokine IFNγ-induced 10 kDa protein (CXCL10), involved in several autoimmune diseases (thyroiditis, rheumatoid arthritis, systemic lupus erythematosus, inflammatory myopathy) exerts detrimental effects at systemic and tissue/cell level; it is reported in association with more severe SSc prognosis [2–4].
Objectives: To compare circulating CXCL10 level in SSc patients starting iloprost (I), prostacyclin analogue with or without sildenafil (S), phosphodiesterase type 5 inhibitor, both vasoactive drugs used in SSc for Raynaud's phenomenon digital ischemic ulcers and/or secondary pulmonary arterial hypertension; SSc subjects under immunosuppressants (DMARDs) or corticosteroids (CCs) - first choice treatments at disease onset - before and after I were also evaluated. Intracellular path activation underlying Th1 cytokine-induced CXCL10 release by human skeletal muscle cells (Hfsmc), endothelial cells (Hfaec), cardiomyocytes (Hfcm) and fibroblasts (hFbs) were compared after iloprost or sildenafil.
Methods: Sera of 27 SSc patients satisfying ACR/EULAR 2013 classification criteria for SSc were analyzed by ELISA before (T0, baseline) and 3 months after I intake (T3) vs. 15 age/gender-matched healthy subjects. Protein extracts from different human cell types were tested by Western blot for IFNγ+TNFα-induced NFkB, Stat1, JNK activation after S or I.
Results: CXCL10 serum level was higher in all SSc under each drug/drug combination (range ~300–500 pg/ml) vs. healthy subjects (~150 pg/ml, $P<0.05$). I intake did not modify baseline CXCL10 in SSc subjects taking only I, DMARDs or CCs, while it significantly decreased CXCL10 in subjects under S (~150 pg/ml, $P<0.05$). In Hfsmc S-induced inhibition of Stat-1/NFkB/JNK phosphorylation was higher vs. I (67/66/66% S vs. 28/30/38% I $P<0.01$). In Hfaec inhibition of Stat1/NFkB phosphorylation was higher and virtually prevented with I (99/92% I vs. 58/66% S $P<0.01$); conversely S-induced inhibition on JNK activation was significantly higher (66% S vs. 20% I $P<0.01$). In Hfcm I-induced inhibition was stronger on Stat1/JNK phosphorylation (67/83% I vs. 33/52% S $P<0.05$/$P<0.01$), while inhibition on NFkB was similar (70% I vs. 71% S). In hFbs, neither S nor I affected IFNγ+TNFα-induced activation of each analyzed path.
Conclusions: Our *in vivo* results show that S and I combination is the more effective in targeting circulating CXCL10 in SSc patients. Our *in vitro* findings show a different inhibitory drug-induced effect onto paths underlying CXCL10 production, depending on cellular/intracellular targets. Thus, we suggest I and S combination as potential pharmacological tool in SSc not limited to treat vascular dysfunction but likely helpful to control different cell/tissue involvement and damage.
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AB0031 IL37 INHIBITS GAG RELEASE FROM HUMAN OA CARTILAGE EXPLANTS

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Background: In healthy cartilage, there is a balance between anabolic and catabolic activities of chondrocytes that maintains the functional integrity of the extracellular matrix. However, during osteoarthritis (OA), chondrocytes become more catabolically active and express increased levels of matrix degrading enzymes, such as MMPs and ADAMTSs. Increased MMP and ADAMTS activity results in a net loss of the extracellular matrix and therefore leads to cartilage damage. Previously, we found that the anti-inflammatory cytokine Interleukin 37 (IL37) is able to counter-regulate the catabolic status of chondrocytes by reducing the IL1β-driven expression of pro-inflammatory cytokines and catabolic enzymes.
Objectives: The goal of this study was to investigate, in human OA cartilage explants, the effect of IL37 on sulfated glycosaminoglycans (GAG) content and synthesis of extracellular matrix molecules and cartilage degrading enzymes to investigate its therapeutic potential in OA.
Methods: Human cartilage was obtained from eighteen OA patients undergoing total knee or hip arthroplasty. Biopsy punches of 4 mm in diameter were made to equalize explant size. After culturing overnight, explants were incubated for 48 h with three doses (1, 10 or 100 ng/ml) of recombinant-human IL37 (rhIL37). In the supernatant of the explant cultures, GAG release was measured with the DMB assay and levels of the ARGS neoepitope, which is one of the products of aggrecan degradation by ADAMTS5, were detected using Western Blot. Furthermore, gene and protein expression of extracellular matrix molecules and cartilage degrading enzymes were measured. Nitric oxide (NO), an important effector molecule that may suppress cartilage matrix synthesis, levels were measured in the supernatant of the explants culture using Griess reagens.
Results: Adding rhIL37 (100 ng/ml) to OA cartilage explants caused a highly significant reduction in GAG release to the supernatant of, on average, 32% in eighteen donors (Figure 1). Gene expression of the matrix molecules aggrecan and collagen type II was not affected, indicating that this effect of rhIL37 was not due to a loss of aggrecan synthesis. Another mechanism to prevent GAG release in cartilage is via inhibition of NO synthesis, but NO levels in the supernatant were comparable between rhIL37 treated groups and the control group. In contrast, after addition of rhIL37, ARGS neoeptiope levels, which reflect ADAMTS5 activity, were dose-dependently down regulated in the supernatant. Furthermore, protein analysis of the explants showed that rhIL37 reduced ADAMTS5 levels. These data indicate that IL37 interferes with the amount active matrix degrading enzymes in the cartilage matrix. However, gene expression of ADAMTS5 was not affected by rhIL37, indicating that the effect of IL37 on ADAMTS5 is post translational.
Conclusions: Our data show that rhIL37 reduces GAG release by OA cartilage explants. The mechanism behind this protective effect of IL37 probably runs via a reduction in ADAMTS5 abundance in the cartilage matrix, which is the main aggrecanase involved in OA This effect of IL37 on ADAMTS5 is probably post translational. Our data indicate that IL37 can maintain cartilage matrix integrity

under OA conditions and is able to reduce the severity of cartilage destruction during OA.

Disclosure of Interest: None declared

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AB0032 UPREGULATION OF CD64 EXPRESSION ON MONOCYTES IN PATIENTS WITH ACTIVE ADULT-ONSET STILL'S DISEASE: A POSSIBLE BIOMARKER FOR ASSESSING DISEASE ACTIVITY

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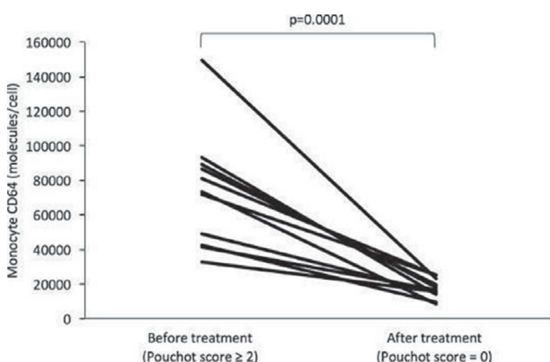
Background: Adult-onset Still's disease (AOSD) is a systemic inflammatory disease of unknown etiology. Overproduction of multiple inflammatory cytokines and subsequent hyperactivation of monocytes/macrophages are prominent characteristics of AOSD. However, there are no convenient and precise methods for evaluating monocyte/macrophage activation in AOSD. We previously reported that monocyte CD64 (mCD64) expression could be quantitatively measured by flow cytometry and its expression was tightly correlated with the activity of systemic lupus erythematosus.

Objectives: We examined the association between mCD64 expression and AOSD disease activity.

Methods: This was a prospective, single-center, observational study conducted between January 2013 and December 2016. Eleven active AOSD patients who fulfilled the Yamaguchi's criteria for AOSD and had the modified Pouchot score of $\geq$2 were enrolled. The mCD64 expression levels were quantitatively measured by flow cytometry and individually assessed both before (Pouchot score $\geq$2) and after treatment (score 0). Other disease-related laboratory data, such as C-reactive protein, ferritin, and white blood cell count, were simultaneously measured. As a control, 16 active systemic lupus erythematosus (SLE) patients (SLE disease activity index $\geq$6), 22 active rheumatoid arthritis (RA) patients (disease activity score with 28-joint counts $>$3.2), and 20 healthy controls (HC) (female, 55%; mean age, 38.7±9.1 years) were enrolled. Statistical analysis was performed by the Mann-Whitney and Wilcoxon-paired tests.

Results: The median mCD64 expression levels were 73,339 [interquartile range (IQR), 45,861–88,181] and 16,443 (IQR, 45,891–88,181) molecules/cell before and after treatment, respectively. Thus, mCD64 expression levels were significantly decreased during the inactive phase compared with those in the active phase in AOSD (p=0.0001). The mCD64 expression levels were significantly higher in patients with active AOSD than in those with active SLE [34,648 (IQR, 44,204–24,657) molecules/cell, p=0.001], active RA [25,167 (IQR, 35,778–22,301) molecules/cell, $p<0.0001$], and in HC [14,174 (IQR, 13,413–17,774) molecules/cell, $p<0.0001$].



Conclusions: These results suggest that mCD64 expression levels are highly upregulated in AOSD and tightly correlated with disease activity. The mCD64 expression level may be a useful biomarker for assessing the disease activity of AOSD.

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AB0033 THE IDENTIFICATION OF IL-17A+, IL-17RA+ AND IL-17RC+ LYMPHOID AND MYELOID CELLS IN BLOOD OF TREATMENT NAÏVE EARLY AND IN SYNOVIAL FLUID OF ESTABLISHED PSORIATIC ARTHRITIS PATIENTS

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Background: Interleukin (IL)-17A is a pro-inflammatory cytokine and is involved in the pathogenesis of psoriatic arthritis (PsA) (1,2). Various cells can produce IL-17A. However, it is not clear which cell types in PsA patients are responsible for the production of IL-17A. In addition, the expression of IL-17RA and IL-17RC on different cell types is not well defined.

Objectives: To identify IL-17A, IL-17RA and IL-17RC positive cells in blood of first diagnosed PsA patients with arthritis and in synovial fluid of established PsA patients with active disease.

Methods: Fresh blood was taken from first diagnosed DMARD and steroid naïve PsA patients (n=10), having arthritis in 1 or more joints (PsA blood). The diagnosis was made by a rheumatologist according to the CASPAR-criteria. In addition, fresh synovial fluid was obtained from established PsA patients (PsA SF) with active disease (n=10) and treated with either methotrexate (n=3) or adalumimab (n=3) or NSAIDs (n=4). Multicolor flow cytometric analysis was performed on PsA blood and PsA SF. For the detection of IL-17A, IL-17RA or IL-17RC the following antibodies were used: IL-17A-PE (eBioscience), IL-17RA or isotype control IgG1k (both Biolegend), IL-17RC or isotype control IgG2b (both R&D systems). The following markers were used to discriminate between different cell populations: T cell subsets (CD3, CD4, CD8, CD45RO, CCR6, TCRγδ), B cells (CD19), NK cells (CD15-CD16+), neutrophils (CD15+CD16+), monocytes (CD33+CD14+CD16+/-), mast cells (CD117+FcER1a+) and eosinophils (CD15+FcER1a+).

Results: Different lymphoid and myeloid cell types were IL-17A positive in PsA blood of first diagnosed PsA patients such as CD3+, TCRγδ+, CD4+, CD8+ lymphoid cells, CD14+ monocytes and eosinophils. In PsA SF of established PsA patients TCRγδ+ T cells, neutrophils, NK cells and eosinophils were IL-17A positive.

In both groups, no difference in expression of IL-17RA and IL-17RC was found on CD4+, CD8+, CD4+CD45RO+CCR6+/-, TCRγδ+ and CD19+ lymphoid cells compared to their isotype control. In contrast, the expression of IL-17RA and IL-17RC was increased compared to their isotype control on neutrophils and monocytes in PsA blood and on neutrophils, monocytes, mast cells and eosinophils in PsA SF.

Conclusions: These preliminary data show that not only lymphoid cells but also specific myeloid cell types may be sources of IL-17A in PsA. Furthermore, not lymphoid cells but IL-17RA/IL-17RC positive myeloid cells such as monocytes, neutrophils, mast cells and eosinophils may be potential target cells for IL-17A. Together, these data suggest a more broad, but specific IL-17A-IL-17RA/RC signaling network between different cell types important in the IL-17A-driven pathogenesis of PsA.

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AB0034 PARAMETERS OF TOTAL BLOOD COUNT; MIGHT THEY BE INDICATORS OF INFLAMMATION IN RHEUMATOID ARTHRITIS AND ANKYLOSING SPONDYLITIS?

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Background: Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are launched as recent markers of inflammation in chronic inflammation principally in cancer and cardiovascular diseases (1–2).

Objectives: Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are chronic inflammatory disorders marked by variable periods of remissions and relapses.Inflammation is most likely the underlying cause in disability, increased comorbidity therefore need to be closely monitored and kept under control (3).For this reason cost effective, accessible and reliable parameters are needed in daily practice. Our aim is to analyze the relation between inflammation and NLR and PLR which are easily calculated from whole blood count parameters.

Methods: Medical records of 425 subjects were analyzed retrospectively.Mean age of the subjects was 44,64±14,07 years (17–89 years),52.9% was female (n=225) and 47.1% was male (n=200).105 of them had RA, 216 of them had AS and 104 were healthy controls.2010 ACR/EULAR classification criteria and modified New York criteria were used for the diagnosis of RA and AS.Erythrocyte sedimentation rate (ESR), C reactive protein (CRP) and whole blood count were recorded with simultaneous DAS28 scores of patients with RA and BASDAI scores of patients with AS.

Results: Hemoglobin levels of RA patients were significantly ($p<0.05$) lower then the levels of control group (p=0.001).ESR, CRP, NLR and PLR were significantly higher then the control group respectively (p=0.001, p=0.001, p=0.001, p=0.040).In AS group hemoglobin, ESR, CRP and NLR values were significantly higher then the control group respectively (p=0.001, p=0.006, p=0.001, p=0.001). No difference was detected between AS and control groups in terms of PLR ($p>0.05$).When patients with high disease activity and patients in remission were compared for both RA and AS groups ESR (p=0.001, p=0.001)