of the surface proteins CD34, CD45, Thy-1 and Podoplanin. The potential effect of cell detachment solutions on the expression of the surface proteins was examined by applying either trypsin or lidocaine. To evaluate on the inflammation status of the FLS, MCP-1 levels in supernatants from FLS in mono-cultures stimulated with either antibodies targeting galectin-9 (Gal-9), an anti-Gal-9 antibody-matched isotype control, LPS or steroid were analyzed by ELISA and compared to culture medium alone. Cell viability were examined using an MTT assay. Data are expressed as mean (95% CI) and analysed by a paired t-test. P-values <0.05 were considered statistically significant.

Results: FLS derived from SFMC were characterized as CD34⁺CD45⁺ and the majority co-expressed Thy-1 and Podoplanin [80.5%, (66.3-94.7%)] confirming the pathogenic phenotype of these cells. This phenotype was not altered by using different methods to detach the cells from the cell-culture plates. FLS receiving anti-Gal-9 antibody treatment showed a significant decreased fold change in MCP-1 secretion (0.84, [0.70;0.98]) compared with unstimulated cells (p=0.036). Treatment with an isotype control did not result in a significant decrease in MCP-1 secretion. This tendency was specific to FLS derived from RA patients as FLS derived from OA patients showed no significant decrease in MCP-1 secretion upon anti-Gal-9 antibody treatment. FLS derived from both RA or OA patients showed a significant increased fold change in secretion of MCP-1 upon LPS stimulation and significantly decreased levels of MCP-1 upon steroid treatment, consistent with the pathogenic phenotype of these cells. None of the different stimulations resulted in morphological changes of the FLS examined by light microscopy. Further, no significant changes in cell viability were detected after anti-Gal-9 antibody treatment.

Conclusion: FLS cultures derived from RA patients at passage 2-5 consist mainly of disease-associated fibroblasts and secrete significantly lower amounts of MCP-1 when treated with an anti-Gal-9 antibody without affecting cell viability. Thus suggesting that Gal-9 neutralization may represent a novel treatment option targeting the stromal environment and inflammation in RA.

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THU0019 INHIBITION OF FUCOSYLATION IN ENDOTHELIAL CELLS REDUCES RHEUMATOID ARTHRITIS ANGIOGENESIS

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Background: Glycosylation has been reported to associate with tumor invasion and metastasis. Fucosylation is involved the biological functions of adhesion molecules and growth factor receptors. In regards to arthritis, we have previously reported that fucosylated proteins were expressed in rheumatoid arthritis (RA) synovial tissues. However, a direct role for fucosylated cytokines in RA has not been demonstrated.

Objectives: To confirm that fucosylated proteins are expressed in RA and are involved in RA angiogenesis.

Methods: Total glycans were determined in serum from normal (NL) subjects and RA patients using mass spectrometry. N-glycans from 10 RA or 10 healthy controls, and 10 pretreatment with tocilizumab (TCZ) or after treatment sera at 24 weeks were purified by glycoblotting using BlotGlycoH. To determine whether fucosylated proteins involved with RA inflammation, the correlation with disease activity score (DAS) 28 (ESR) was measured. 2-deoxy-D-galactose (2-dGal) is an analog of hexose that inhibits fucosylation. In order to confirm the role of fucosylation in RA angiogenesis, we did Matrigel assays *in vitro*. To block the expression of fucosylated proteins, human umbilical vein endothelial cells (HUVECs) were treated with 2-dGal (15 mM) for 5 days. After treatment with 2-dGal, HUVECs were plated on Matrigel and were incubated with phosphate buffered saline (PBS) or RA synovial fluids. In addition, chemotaxis assays were performed to determine the role of fucosylation in HUVEC migration. Finally, expression of proangiogenic cytokines such as

fractalkine/CX3CL1, CXCL16, interleukin (IL)-8/CXCL8, monocyte chemotactic protein 1 (MCP-1)/CCL2, epithelial neutrophil-activating protein 78 (ENA-78)/CXCL5 and vascular endothelial growth factor (VEGF) in 2-dGal treated HUVEC conditioned medium were measured by ELISA.

Results: Total glycans in RA serum were significantly higher than in NL serum [mean ± SEM; 477 ± 24 pmol/μl (n=10) and 339 ± 14 pmol/μl (n=10), p<0.05, respectively]. In addition, total glycans in RA serum were significantly decreased with tocilizumab treatment at 24 weeks. Total glycans in RA serum were also correlated with DAS28 (ESR). Percent of fucosylated proteins in total glycans were decreased with TCZ treatment at 24 weeks. 2-d Gal treated HUVEC tube formed towards RA synovial fluids (n=6 patients) were decreased compared with nontreated HUVEC tube formed (number of tube formed; 7 ± 1 and 25 ± 2, p<0.05, respectively). We found that 2dGal treated HUVECs showed decreased migration compared with nontreated HUVECs, towards RA SF (numbers of HUVEC migrated; 3 ± 1 and 34 ± 4, n=5 patients, p<0.05). Fractalkine/CX3CL1, CXCL16, or IL-8/CXCL8 in 2-d Gal treated HUVEC conditioned medium were decreased compared with in non-treated HUVEC conditioned medium but not MCP-1/CCL2, ENA-78/CXCL5, or VEGF.

Conclusion: These data indicate that glycoproteins are involved with RA, and play a role in angiogenesis in RA and suggest that targeting glycosylation especially fucosylation may provide a method by which to decrease inflammation and potentially treat other inflammatory diseases.

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THU0020 COMPARATIVE STUDY OF THE PHOSPHODIESTERASE TYPE 5 INHIBITOR SILDENAFIL AND THE PROSTACYCLIN ANALOGUE ILOPROST ON IP10 MODULATION IN SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune disease ending in multiorgan fibrosis. Vascular damage, responsible for the vascular alteration and disruption, has been suggested to play a key role in disease maintenance and progression [1]. Recent data demonstrated that high serum levels of the interferon (IFN)-γ-induced protein 10 (IP-10) in SSc patients correlated with peripheral vascular injury and increased in association with nailfold capillaroscopic pattern worsening and digital ulcers presence [2, 3]. Between the vasoactive drugs used for SSc treatment, the prostacyclin analogue iloprost (I) and PDE-5 inhibitor sildenafil (S) seem to have high vasodilatory and immunomodulatory actions [4-6].

Objectives: To investigate and compare the ability of S and I to modulate: IP-10 circulating levels in SSc patients under different treatments; IP-10 release by human endothelial (Hfaec) cells subjected to Th1-related inflammatory stimuli.

Methods: Sera of 28 patients satisfying ACR/EULAR 2013 classification criteria for SSc were analyzed by ELISA. IFNγ+TNFα-induced activation of NFκB, STAT1, JNK, ERK1/2 and AKT in Hfaec after S or I was tested by Western blot.

Results: The treatment with S significantly reduced IP-10 serum levels vs. treatment with (DMARDs) and corticosteroids (CCs) (184.1±65.10 vs. 880.9±339.0 pg/ml and vs. 426.5±101.7, respectively, P<0.01); while no significant difference has been found vs. I (184.1±65.10 vs. 282.7±46.6 pg/ml). In Hfaec, S and I differently counteracted the IFN-γ/TNF-α-induced phosphorylation of JNKs (respectively 61.6±20.1% and 9.5±13.1% of phosphorylation vs. I+T-induced taken as 100%), STAT1 (respectively 49.2±15.8% and 93.4±1.2% of phosphorylation vs. I+T-induced taken as 100%), NFκB (respectively 72.6±8.3% and 92.2±3.3% of phosphorylation vs. I+T-induced taken as 100%), ERK1/2 (respectively 31.6±7.9% and 27.3±4.4% of phosphorylation vs. I+T-induced taken as 100%), and AKT (respectively 85.7±5.6% and 3.8±7.4% of phosphorylation vs. I+T-induced taken as 100%).

Conclusion: *In vivo* results show that S and I have more effect in targeting serum IP-10 in SSc patients than other therapeutic treatments. *In vitro* data show different inhibitory drug-induced effects onto paths underlying IP-10 production, depending on intracellular targets. S could be a potential pharmacological tool as effective as I to control IP-10 in blood or at endothelial cell level in SSc.

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THU0021 CLINICAL SIGNIFICANCE OF WEIGHT LOSS ON THE LEVEL OF PROINFLAMMATORY CYTOKINES IN PATIENTS WITH OSTEOARTHRITIS

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Background: Obesity is a condition that prolongs chronic inflammation and promotes synthesis and secretion of pro-inflammatory factors by adipose tissue, such as classical cytokines, tumor necrosis factor- α (TNF- α), adipokines (leptin, adiponectin, resistin) and other newly identified proinflammatory factors (hemerin, lipokain, serum amyloid protein 3) [1,2,3,4,5]. Nowadays one of the most actively studied adipokines is nicotinamide phosphoribosyltransferase (visfatin, Nampt).

Objectives: We investigated the relationship the effect of weight loss over 5 kg on the clinical manifestations of OA and Nampt serum levels in patients with OA.

Methods: We observed 160 patients with different forms of OA ranged in age from 36 to 78 years, of whom there were 104 (65%) women (mean age 52.08 $\pm$ 1.58 years), and 56 (35%) of men (mean age - 54.07 $\pm$ 2.0 years) and the control group (60 healthy persons) with no complaints of pain in the joints over a lifetime, and without clinical signs of OA. Nampt level in serum was determined by ELISA using a commercial test systems.

Results: As overweight patients were recruited in the study, hypocaloric diet low in animal fats and physiotherapy has been recommended to all participants. The positive dynamics in body weight loss over 5kg within 3 months has been achieved by 36 patients (23%). All patients were divided into two groups to study the effect of weight loss on the clinical manifestations of OA. These data proves that obesity may be an important risk factor for OA progression. As a result, weight loss results in decreasing metabolic disorder severity. In the second group of patients we have seen a decrease in all the parameters, but a significant difference has been observed only in the level of CRP, level of pain at rest and during walking according to VAS scale and total index on the WOMAC. However, patients with weight loss over 5 kg had significantly greater positive dynamics of clinical parameters than in the second group without weight loss. This fact is probably explained by the decreased activity of inflammatory process after OA therapy and weight reduction.

Conclusion: Thus, as a result of our study patients with OA with weight loss of more than 5 kg had more obvious pain relief than patients with the original weight. These findings suggest that there is a possible role of visfatin in the pathogenesis of osteoarthritis. All patients with OA with a BMI over 25 kg/m² are recommended to lower their weight to decrease the mechanical stress on the joints, and also to reduce the severity of inflammation and metabolic disorders.

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THU0022 ETANERCEPT INCREASES AUTOPHAGY AND REDUCES APOPTOSIS INDUCED BY TNF- α EXPOSURE IN ENDOTHELIAL CELLS IN VITRO

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Background: Rheumatoid arthritis (RA) is associated with high incidence of cardiovascular events, mainly due to accelerated atherosclerosis and endothelial dysfunction (1). Apoptosis in endothelial cells has been associated with vascular wall damage, contributing to atherosclerotic plaque formation (2). At the contrary, autophagy may play a protective role in preventing development of atherosclerosis. However, the exact mechanism which controls the autophagy of endothelial cells is still unknown. Tumor necrosis factor alpha (TNF- α) is one of the leading cytokines involved in the pathogenesis of RA. Several evidences suggest a pro-atherogenic effect of TNF- α in patients with RA. Etanercept (ETA) and other TNF- α inhibitors are effective treatments for joint inflammation in RA, but their effect on endothelial dysfunction is still not well understood.

Objectives: The aim of this study was to investigate *in vitro*, the effects of ETA treatment on endothelial cells exposed to TNF- α , with particular attention on apoptosis levels and autophagy pathway.

Methods: *In vitro* effects of ETA and TNF- α on endothelium were evaluated using human umbilical vein cell line EA.hy926. Cells were treated with ETA (15 μ g), TNF- α (10 ng/ml), alone or in combination. An untreated control cell culture was also performed. After 24 hours, apoptosis levels and autophagy have been evaluated. Apoptosis was analyzed by flow cytometry using a FITC-conjugated annexin V and propidium iodide apoptosis detection kit (3); autophagy was analyzed by western blot for the expression level of the autophagic markers LC3-II and p-62 (4).

Results: TNF- α treatment significantly increased apoptosis in endothelial cells compared both to untreated and ETA-treated cells ($p=0.0067$ and $p=0.00187$ respectively) [Figure 1]. The co-treatment with TNF- α and ETA significantly reverted the increase of apoptosis levels ($p=0.001$ versus TNF α alone). Both ETA and TNF α treatments significantly increased the expression level of LC3II in treated cells when compared to untreated cells ($p=.18$ and $p=.3$ respectively). The co-treatment induced an additive effect on autophagy levels ($p<0.0001$ versus untreated cells). At last, the expression level of p-62 showed an inversely proportional trend to LC3II levels [ETA ($p=0.0001$), TNF- α $p=.1$), ETA+TNF- α $p=.5$) versus untreated cells, respectively] [Figure 2].

Conclusion: Our results show that treatment with Etanercept have a protective effect on endothelial cells *in vitro*, reversing the apoptosis induced by TNF- α . Moreover, this is the first study that supports a possible effect of Etanercept in increasing autophagy level in endothelial cells. Reducing TNF α -induced apoptosis, Etanercept treatment may lead to an improvement of endothelial function and to a reduction of accelerated atherosclerosis.

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