

We revealed a correlation between IgG aCL, IgG $\alpha\beta$ -GP1, IgG aAnV and TNF- α , IgG aCL and IL-6 in SLE patients, and only one between IgG aAnV and hs-CRP in RA patients. There wasn't any correlation between aPL and inflammatory mediators in the control group.

Univariate analysis has demonstrated an association of IgG aAnV with IMT ($r=0,320$, $p=0,044$) in SLE patients and positive association between TNF- α and IMT ($r=0,362$, $p=0,028$) in RA patients. Furthermore, we found an association between IL-6 and IgG aPT ($r=0,426$, $p=0,038$), TNF- α and IgG aCL ($r=0,419$, $p=0,042$) in SLE patients with carotid atherosclerosis. There wasn't any association between investigated parameters in the control group.

Conclusions: The association between inflammatory mediators and disease activity has been confirmed in ARD patients. Increased autoimmune activity has been verified both in patients with SLE and RA. It has been determined that IgG aAnV had more significance for IMT in patients with SLE, TNF- α - in RA patients. Our data can suggest that inflammatory mediators and antiphospholipid antibodies are involved in the atherosclerotic process in patients with ARD.

Disclosure of Interest: None declared

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AB0049 NF- κ B-INDUCING KINASE REGULATES LT β R-DRIVEN NF- κ B SIGNALING AND INFLAMMATORY ACTIVATION OF ENDOTHELIUM

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Background: Sites of chronic inflammation, such as rheumatoid arthritis synovial tissue, are characterized by neovascularization and often contain tertiary lymphoid structures with characteristic features of lymphoid organs such as endothelial venules (HEV), and sometimes even true germinal centers. Ligation of the lymphotoxin (LT)- β receptor (LT β R) results in activation of both canonical and NF- κ B-Inducing Kinase (NIK)-dependent non-canonical NF- κ B signaling in endothelial cells (ECs) and plays a crucial role in lymphoid neogenesis. Non-canonical NF- κ B signaling in ECs promotes inflammation-induced angiogenesis and triggers the development of the cuboidal HEV appearance. However, the relative contribution of the individual pathways to the acquisition of leukocyte traffic-regulating properties by ECs is less well understood.

Objectives: To identify the molecular pathways by which LT β R drives inflammatory activation of ECs to promote interactions with leukocytes.

Methods: Primary human ECs were treated with LT β or LIGHT to activate LT β R. Induction of downstream signaling pathways was assessed by western blot analysis and NF- κ B transcription factor ELISA. The expression of adhesion molecules, inflammatory cytokines and chemokines, such as CXCL1, CXCL5, CXCL8 and GM-CSF in ECs was measured by RT-qPCR and cytokine antibody arrays. EC interactions with leukocytes were determined by an adhesion assay, and EC barrier integrity was assessed by a permeability assay. To repress canonical NF- κ B signaling pathway, a small molecule inhibitor of IKK β was used, and inactivation of non-canonical NF- κ B signaling was achieved with siRNAs targeting NF κ B2. The role of NIK in LT β R signaling was investigated using small molecule inhibitors of NIK, siRNAs targeting NIK and adenoviral vectors encoding wild type and kinase-deficient NIK.

Results: LT β R triggering in ECs resulted in activation of both canonical and non-canonical NF- κ B signaling pathways and induced the expression of inflammatory cytokines and chemokines (CXCL1, CXCL5, CXCL8, MCP-1, GM-CSF, CCL5). Consistent with inflammatory activation of ECs, LT β R ligation also induced adhesion of immune cells to activated endothelium and increased permeability across EC monolayers. IKK β inhibition completely repressed LT β R-induced inflammatory activation of ECs, indicating that this process was mediated through canonical NF- κ B signaling. Interestingly, inactivation of NIK with small molecule inhibitors and siRNAs significantly decreased LT β R-induced expression of inflammatory cytokines and adhesion of immune cells to endothelium, whereas silencing of NF κ B2 had no effect. This suggests that the non-canonical pathway is dispensable for NIK-dependent activation of endothelial cells through the canonical NF- κ B pathway. Further analyses, including silencing of NIK and NIK overexpression, demonstrated a role for NIK in activation of the canonical NF- κ B pathway by amplifying IKK complex activity.

Conclusions: These findings suggest that in addition to its pivotal role in the non-canonical pathway, NIK can serve as an amplifier of the canonical NF- κ B pathway and associated inflammatory responses in ECs mediated by LT β R ligation, which may play a role in development and maintenance of chronic inflammation.

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AB0050 IMPAIRED ADIPONECTIN AND LEPTIN LEVELS DURING OSTEOARTHRITIS ONSET AND DEVELOPMENT IN STR/ORT MICE

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Background: Obesity is a risk factor for osteoarthritis (OA). In obese subjects

OA develops not only in weight-bearing joints but also in non-weight-bearing joints, suggesting that dysregulated metabolism in obese patients may promote OA onset.

As obesity evolves many physiological parameters are dysregulated, including the levels of adipokine hormones such as leptin and adiponectin. For this reason, it has been suggested that adipokine levels in serum and synovial fluid are associated with a worsening of synovial inflammation and OA progression in these patients⁽¹⁾.

In vivo and in vitro studies show that high levels of leptin induce the synthesis of metalloproteases involved in cartilage degradation⁽²⁾. Conversely, dietary-induced weight loss is associated with increased adiponectin serum levels and reduced loss of tibial and femoral cartilage volume, suggesting a protective role of adiponectin in OA.

STR/ort mice are an animal model of spontaneous OA characterized by early pathology development (at about 20 weeks) and dysregulated metabolism⁽³⁾. Notably, these mice have adiponectin serum levels lower than those found in control mouse strains⁽⁴⁾.

Objectives: To evaluate whether adiponectin and leptin serum levels are associated with OA development and/or progression in STR/ort mice.

Methods: First, we measured the time course of adipokine levels in STR/ort mice before the onset of OA (at 8, 14 and 20 weeks of age), and in age-matched CBA control mice. Then, we calculated the ratio leptin/adiponectin (L/A) in the serum of STR/ort mice during OA progression (at 20, 30 and 40 weeks). Blood samples were collected from caudal vein (time course) or from vena cava at sacrifice, when knee joints were collected, processed for histology and blindly scored according to OARSI and Mankin's methods.

Results: Adiponectin serum levels in STR/ort mice at 8, 14 and 20 weeks were significantly lower than in age-matched CBA mice. Instead, leptin serum levels in STR/ort mice were higher than in CBA strain at 14 and 20 weeks. Consequently, there was a relevant difference in the ratio L/A between the two strains, with greater L/A values in STR/ort mice at 14 and 20 weeks. (Table 1)

In STR/ort mice, the ratio L/A tended to further increase between 30 and 20 weeks (1.73 ± 0.16 from 1.28 ± 0.17 , respectively), in parallel with the increase in OARSI scores of knee joints (11.1 ± 1.5 vs 8.4 ± 1.3). The histopathological score increased in STR/ort mice even between 30 and 40 weeks, but without a concomitant increase in the ratio L/A.

Adipokines levels (mean \pm SEM)			
Age (Weeks)	8	14	20
CBA (n=10)			
Adiponectin (μ g/ml)	20.2 \pm 0.7	16.0 \pm 0.4	14.5 \pm 0.3
Leptin (ng/ml)	1.5 \pm 0.3	2.5 \pm 0.3	3.1 \pm 0.4
L/A	0.07 \pm 0.02	0.16 \pm 0.02	0.21 \pm 0.03
STR/ort (n=14)			
Adiponectin (μ g/ml)	9.2 \pm 0.3**	7.3 \pm 0.2**	6.0 \pm 0.2**
Leptin (ng/ml)	3.3 \pm 0.5	8.7 \pm 1.3**	7.9 \pm 1.1**
L/A	0.36 \pm 0.05	1.15 \pm 0.16**	1.28 \pm 0.17**

** $p < 0.001$ STR/ort vs CBA

Conclusions: We show for the first time that leptin serum levels and the ratio L/A in STR/ort mice are higher than in CBA mice, and that the ratio L/A in STR/ort mice increases as their histopathological scores worsen. We suggest that dysregulated levels of these adipokines may be associated or even precede OA development in this animal model.

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AB0051 INTERLEUKIN-6 BLOCKADE WITH TOCILIZUMAB DECREASES METALLOPROTEINASE-9 ACTIVITY IN SYNOVIAL FIBROBLASTS STIMULATED WITH SYNOVIAL FLUIDS OF PATIENTS WITH RHEUMATOID ARTHRITIS OR SPONDYLOARTHRITIS

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Background: Fibroblast-like synoviocytes (FLS) exhibit a transformed aggressive phenotype characterized by increased secretion of pro-inflammatory cytokines and matrix metalloproteinases (MMPs). Early pathological mechanisms that explain the change to an altered phenotype in FLS of chronic inflammatory arthropathies remain largely unknown. The composition of synovial fluids (SF) is very complex and strongly influences the microenvironment of joints including FLS thus representing an inseparable element of the disease. The MMP-9 is a

gelatinase expressed in three major forms: dimer, monomer and a complex with neutrophil gelatin-associated lipocalin (NGAL). Interleukin-(IL)-6 is a pleiotropic cytokine expressed by a variety of immune and non-immune cells. However, the mechanisms by which IL-6 contributes to the pathogenesis of chronic arthropathies are not fully understood.

Objectives: The purpose of the present work was to perform a comparative study of the IL-6 production and MMP-9 activity in FLS stimulated with SF from patients with osteoarthritis (OA), rheumatoid arthritis (RA) or spondyloarthritis (SpA). In addition, the effect of IL-6 blockade on MMP-9 activity was evaluated.

Methods: Primary FLS were obtained from SF of the RA patients. Furthermore, the SW982 human synovial cell line was used. The SF of patients with OA (n=11), RA (n=11) or SpA (n=9) patients were pooled. The FLS were stimulated with OA, RA or SpA SF pools and supernatants (SN) were collected after 24, 48 and 72 h. The IL-6 levels were assessed in the SN by ELISA. The gelatinase activity of the SN was determined by zymography. The IL-6 function was blocked with the anti-IL-6 receptor antagonist tocilizumab (TCZ) (200µg/ml).

Results: Earlier induction of IL-6 in SW982 cell line was observed by RA and SpA SF stimulation since significant levels were detected at 24 h ($p < 0.001$ and $p < 0.01$ compared with non-stimulated cells, respectively), whilst OA SF induced significant IL-6 secretion at 72 h ($p < 0.01$). Similar results were observed in primary FLS. In contrast to SF of OA patients, SF of patients with RA or SpA induced increased and sustained secretion of active MMP-9. Moreover, the molecular weight band corresponding with NGAL-MMP-9 complex, considered a protected form of MMP-9, was detected with higher intensity in the SN of FLS stimulated with RA or SpA SF compared with OA SF ($p < 0.001$). In the presence of TCZ, significant inhibition in the gelatinase activity of all MMP-9 forms was observed at 48h of stimulation with RA or SpA SF ($p < 0.001$ for MMP-9 dimer and NGAL-MMP-9 complex; $p < 0.01$ for MMP-9 monomer, compared with FLS stimulated in absence of TCZ).

Conclusions: We conclude that SF of patients with inflammatory arthritis recreate a differential microenvironment for FLS that impacts on early phenotypic changes of these cells. The IL-6 provokes augmented and persistent MMP-9 activity in FLS stimulated with RA or SpA SF. This work identifies TCZ as an inhibitor of all forms of MMP-9.

Disclosure of Interest: None declared

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AB0052 INCREASED PERIPHERAL CD8+ T CELL RESPONSES IN SLE BY LOW-DOSE IL-2 TREATMENT

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Background: CD8+ T cell responses to viral pathogens is crucial for the prompt resolution of acute infections. SLE patients are more likely to have infections due to suppression of immune system by long-term glucocorticoid and immunosuppressive agent intake. Our previous study showed that low-dose IL-2 is effective in SLE.

Objectives: The present study is to evaluate the potential anti-infection effect of low-dose IL-2 in refractory SLE patients.

Methods: Nine refractory SLE patients and 9 health controls (HCs) were recruited three cycles of 1 million IU recombinant human IL-2 (rhIL-2), administered subcutaneously every other day for 2 weeks, followed by a 2-week break. The disease activities were evaluated by rheumatologist. The frequencies of T cell subsets were assayed by flow cytometry. Virus-specific CD8 T cells responses were determined based on TNF- α , IFN- γ and Granzyme B producing CD8 T cells upon CMV-EBV-Flu (CEF) viral peptide pool stimulation and subsequent intracellular staining.

Results: Most patients showed good clinical responses after three cycles of low-dose IL-2 treatment. Clinical improvement was observed in SIR-4 response, improved complement 3 and 4 serum level and decreased anti-ds-DNA serum level. Functional profiling of CD8 T cells in low-dose IL-2 treated patients revealed an increased in the frequencies of CEF viral peptide specific TNF- α and Granzyme-B⁺ CD8 T cells. Moreover, low-dose IL-2 treated patients showed stronger antigen-specific response demonstrated by an increased stimulated/non-stimulated TNF- α -producing CD8 T cells proliferation fold. Compared with HCs, SLE patients showed significantly lower frequencies of CEF specific Granzyme-B producing CD8 T cell, and treatment with low-dose IL-2 significantly increased the frequency of these Granzyme-B⁺ CD8 T cells in SLE patients.

Conclusions: Low-dose IL-2 treatment was effective and safe in refractory SLE patients. Virus-specific antigen-specific CD8 T cell response could be enhanced upon this treatment which might be potentially valuable in anti-infection in SLE.

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AB0053 MANGIFERIN MODULATES TNF-ALPHA AND MMP-9 SIGNALING

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Background: Rheumatic manifestation such as arthritis, spondylitis are the most common frequent extra-intestinal manifestations of inflammatory bowel disease (IBD), affecting 6 to 46% of patients. IBD is an umbrella term with two most prevalent entities namely Crohn's disease and Ulcerative colitis, defined as idiopathic chronic, relapsing and remitting inflammatory condition of intestinal tract. MMP-9, a matrixin is involved in the degradation of extracellular matrix promoting inflammation. Studies on MMP-9 knockout mice illustrated protection from collagen induced rheumatoid arthritis (RA) and Dextran sulphate sodium (DSS) induced colitis. Furthermore elevated MMP9 levels can be found in the synovial fluid of RA patients as well as IBD patients signifying that targeting MMP9 may have therapeutic importance. Mangiferin, an active component of Mango has demonstrated anti-inflammatory effects in the treatment of rheumatoid arthritis with low side-effects^[1,2]; but its usefulness in chronic remission and relapse phases of human IBD i.e. chronic DSS model have not screened till date.

Objectives: We investigated the therapeutic potential of Mangiferin; in a clinical relevant chronic model of DSS induced colitis in mice.

Methods: Female BALB/c mice (8 to 12 wks) were randomized into four groups. Colitis was induced by cyclical administration of 5% DSS to mice i.e. 3 cycles of DSS with every cycle consisting of 7 days DSS followed by 7 days of autoclaved drinking water (7D DSS + 7D water). Group I (Normal control): free access to autoclaved drinking water. Group II (DSS control): free access to 5% DSS. Group III (DSS + Mangiferin_30mg/kg): free access to 5% DSS + oral Mangiferin at 30mg/kg. Group IV (DSS + Mangiferin_60mg/kg): free access to 5% DSS + oral Mangiferin at 60mg/kg. Mangiferin treatment was initiated following second cycle of DSS (i.e. Day 21); after assuring that colitis relapsed in mice. One fragment of the colon was fixed in 10% neutral buffered formalin for microscopic examination while the remaining tissue was divided into parts and stored at -70°C for assessment of biochemical markers of oxidative stress and inflammatory cytokines such as TNF- α , IL-1 β , MMP-9.

Results: Mangiferin treatment ameliorated the *clinical parameters* (body weight loss, stool consistency, occult blood), reduced *microscopic damage* (re-established mucosal architecture, abridged neutrophil infiltration), restored *epithelial barrier integrity* (diminished goblet cell loss), attenuated biochemical markers of *oxidative stress* (GSH, CAT, SOD, MDA, MPO), crucial *inflammatory cytokines* TNF- α , IL-1 β and attenuates *MMP-9* levels implicated in the pathogenesis of arthritis and IBD.

Conclusions: Considering the beneficial effects of Mangiferin in arthritis and IBD, we suggest that it would be valuable to use Mangiferin in IBD patients with arthritis as its extra-intestinal manifestation.

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AB0054 CXCL4 POTENTIATES TLR-DRIVEN POLARIZATION OF HUMAN DENDRITIC CELLS TOWARDS CYTOKINE PRODUCTION, ANTIGEN CROSS-PRESENTATION AND INCREASES STIMULATION OF CD8+ T-CELLS

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Background: CXCL4 is a chemokine produced by activated platelets and immune cells. Several studies have reported that CXCL4 plays a critical role on physiological processes since it affects the proliferation and maturation of megakaryocytic and hematopoietic progenitor cells, regulates coagulation and wound healing, displays anti-tumoral and angiostatic activity and induces immune cell modulation. Dysregulation of these processes causes the disturbance of the immune system and homeostasis, and might lead to pathological conditions. Indeed, a strong correlation was previously found between elevated CXCL4 levels in the circulation and the clinical features of patients with systemic sclerosis (SSc) (1). Dendritic cells are essential players in innate defence and bridging towards adaptive immune responses, thereby contributing to both immune activation and maintenance of homeostasis.

Objectives: Considering previous observations on the association of dendritic cells and T-cell dysfunction in SSc, we here investigated the effect of CXCL4 on monocyte-derived DC (moDC) differentiation, on Toll-like receptor (TLR)-mediated responses and on activation of polyclonal and antigen-specific CD8⁺T-cells.