4 weeks): (I) 8 week old mice with established inflammatory, erosive arthritis and (II) 12 week old hTNFtg mice with severe inflammatory, erosive arthritis (n=8–10 animals). Clinical signs of arthritis were weekly assessed. Knee and ankle joints were used for μ CT (Scanco μ CT35) and subsequent histology. Toluidine blue staining indicated proteoglycan contents, tartrate-resistant acid phosphatase staining identified osteoclasts. Immunohistochemical stainings were performed for collagen type II (col II) and osterix. Expression of chondrogenic markers was analysed from RNA paw extracts.

Results TNF blockade significantly improved clinical signs in both treatment cohorts. Compared to eroded bone surfaces at week 8 (before treatment), TNF blockade led to improved bone architecture with regular, smooth bone surfaces in CT scans from knee and ankle. Histologically, treatment allowed complete resolution of synovitis, osteoclast activity and subchondral bone erosions. Despite sites of intact bone tissue, regenerative processes of cortical and subchondral bone were found. Erosions of bone and calcified cartilage were filled with col II positive cartilaginous or fibro-cartilaginous tissue. Hyperproliferative chondrocytes and endochondral ossifications were found in knees. Consistently, chondrogenic markers like Sox9 transcription factor and col II were markedly increased, accompanied by decreased expression of cartilage degrading enzymes (MMP3, MMP13) upon treatment. Of note, menisci and patella tendon showed massive hyperproliferative chondrocytes, col II and osterix staining.

Anti-TNF treatment at later stages with severe bone destruction (at week 12) could not completely resolve synovitis and restore structural damage. Pannus tissue transformed into chondrocyte rich, fibrocartilaginous tissue. Callus and ossifications were formed in knees.

Conclusions Therapeutic TNF blockade of inflammation-mediated joint damage enables the initiation of directed but also undirected regenerative processes. Remission of synovitis seems to be essential to promote bone and calcified cartilage repair.

Disclosure of Interest None declared.

P115/019 IMMUNOMODULATORY ACTIVITY OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS OF ANKYLOSING SPONDYLITIS PATIENTS

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Career situation of first and presenting author Post-doctoral fellow.

Introduction Ankylosing spondylitis (AS) is a chronic autoimmune disease characterized by inflammation and pathological new bone formation at axial joints with resulting spinal segments fusion. The immune abnormalities observed in AS patients concern mainly T lymphocytes and manifest by increased numbers of circulating Th17 cells and defects of regulatory T (Treg) cells. Adipose mesenchymal stromal/stem cells (ASCs) exert

immunosuppressive effects on different immune cells, including T lymphocytes, and may be promising option for successful therapy of AS.

Objectives In this study we have focused on immunoregulatory activity of ASCs from AS patients (AS/ASCs) toward healthy donor peripheral blood mononuclear cells (PBMCs) and T lymphocytes with special regard to Th17 and Treg cell subsets.

Methods AS/ASCs of 15 AS patients and commercially available ASC lines from healthy donors (hASC) were used. ASCs were co-cultured with either anti-CD3/CD28-stimulated CD4⁺ T lymphocytes or mitogen-stimulated PBMCs of allogeneic healthy volunteers. Expression of CD25 and FoxP3 transcription factor in T cells and these cells proliferation were evaluated by flow cytometry. Concentrations of cytokines associated with Th1 (IFN γ), Th17 (IL-17AF) or Treg (IL-10, TGF β) cells as well as of factors related to immunomodulatory function of ASCs (PGE₂, kynurenines) were measured by ELISAs in co-culture supernatants.

Results In the co-cultures of ASCs with PBMCs both hASCs and AS/ASCs exerted similar inhibitory effect on the proliferation response of T CD4⁺ and CD8⁺ cells. Similar results were obtained in co-cultures of ASCs with purified T CD4⁺ cells. In the co-cultures of hASCs and AS/ASCs with PBMCs as well as with purified T CD4⁺ cells the significant increases of kynurenines and PGE₂ production were observed. In co-cultures of ASCs and PBMCs there was a marked decrease of IFN γ and significant increase of IL-17AF. Concomitantly, Treg proportion in these co-cultures was elevated. In contrary, in the co-cultures of activated T CD4⁺ cells with ASCs there was some increase of IFN γ and IL-17AF whereas Treg proportion was downregulated.

Conclusions AS/ASCs and hASCs exert comparable effects on T cells. Since different effects were observed in the co-cultures of ASCs with PBMCs than with CD4⁺ cells, it seems that ASCs activity toward Th17 and Treg cells is dependent on the presence of accessory cells.

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Disclosure of Interest None declared.

P116 BASIC CHARACTERISTICS OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS OF ANKYLOSING SPONDYLITIS PATIENTS

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Career situation of first and presenting author Post-doctoral fellow.

Introduction Application of mesenchymal stem/stromal cells (MSCs), endowed with immunosuppressive and regenerative properties, may be promising option for successful therapy of ankylosing spondylitis (AS) patients, characterised by inflammation and pathological bone remodelling. Adipose tissue is an

easily accessible, rich source of MSCs. Biology of MSCs in AS is poorly understood and data describing MSCs from adipose tissue (ASCs) are missing.

Objectives The aim of this study was to perform comparative basic characterisation of ASCs of AS patients (AS/ASCs) and ASCs line originating from healthy volunteers (hASCs).

Methods AS/ASCs of 15 AS patients and commercially available hASC lines were used. Phenotype and expression of adhesion molecules (ICAM-1, VCAM-1) on ASCs were evaluated by flow cytometry. Basal and cytokine (tumor necrosis factor +interferon γ or interleukin-23)-induced secretion of nine factors (TGF β , IL-6, galectin, LIF, IL-1Ra, PGE $_2$, sHLA-G, TSG6 and kynurenines) known to mediate immunomodulatory activity of MSCs was measured by ELISAs. ASCs were co-cultured with either anti-CD3/CD28-stimulated CD4 $^+$ T lymphocytes or mitogen-stimulated peripheral blood mononuclear cells (PBMCs) of allogeneic healthy volunteers. Expression of activation markers (HLA-DR, CD25, CD69) on T cells and these cells proliferation were evaluated by flow cytometry.

Results There were no significant differences in the phenotype, expression of adhesion molecules and secretory potential between hASCs and AS/ASCs. Inhibition of T cell proliferation was found in both co-culture systems, while modulation of activation markers expression (CD25 down-regulation, CD69 up-regulation) on CD4 $^+$ T and CD8 $^+$ T cell subsets was stated in ASCs-PBMCs co-cultures only and hASCs and AS/ASCs exerted similar effects.

Conclusions AS/ASCs have phenotype and basic biological features (i.e. secretory potential, some attributes related to immunomodulatory activities) comparable to hASCs.

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Disclosure of Interest None declared.

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VISFATIN DOWN-REGULATES GROWTH PROMOTING LNCRNA H19 DURING OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STROMAL CELLS

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Career situation of first and presenting author Student for a master or a PhD.

Introduction Osteoarthritis (OA) and osteoporosis are destructive bone diseases causing chronic pain and leading to disability. Mesenchymal stromal cells (MSC) are essential to bone health and tissue repair. Adipokines such as visfatin alter the osteogenic potential of MSCs and contribute to the loss of bone homeostasis. Long non-coding RNA H19 is one of the first lncRNAs discovered and relevant for distinct processes, e.g. during embryonic growth and tumor formation. lncRNAs interact directly with DNA, RNA as well as proteins, modulating transcription, protein expression and protein function. H19 upregulation was shown in osteogenic differentiation of MSCs.¹ H19 increases the osteogenic potential via TGF β 1 and Wnt/ β -catenin pathways.^{2, 3}

Objectives To investigate the influence of visfatin on H19 expression during osteogenesis.

Methods Human MSCs from healthy donors (hMSC) and primary human MSCs from osteoarthritis patients (phMSC) after knee replacement surgery were treated with differentiation medium to induce osteogenic differentiation (OD). Matrix mineralization (MM) was quantified after 21 days by Alizarin Red. H19 expression by realtime PCR and IL-6 production by ELISA were measured.

Results lncRNA H19 was up-regulated during OD. Although the H19 upregulation was not altered by co-stimulation with resistin, leptin or TNF α , visfatin co-stimulation during OD down-regulated H19 expression up to 10-fold as compared to unstimulated MSCs. The effect was significant in phMSCs at two of three measured time points (day 7 $p=0.03$; day 14 $p=0.002$, $n=3$) and in hMSCs at day 14 ($p=0.0003$, $n=4$). Visfatin co-stimulation of MSCs in OD increased MM, as well as IL-6 levels. However, TNF did not alter H19 expression or increase MM.

Conclusions Visfatin co-stimulation during osteogenesis down-regulated lncRNA H19 expression, indicating a loss of the growthpromoting effects of lncRNA H19 in affected areas of destructive bone disease. This regulatory effect was specific to visfatin and did not occur upon co-stimulation with other adipokines or inflammatory stimuli such as TNF supporting a TNF-independent effect of visfatin.

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Disclosure of Interest None declared.

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PODOPLANIN REGULATES THE MIGRATION OF MESENCHYMAL STROMAL CELLS AND THEIR INTERACTION WITH PLATELETS

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Career situation of first and presenting author Young investigator.

Introduction Within the rheumatoid joint, mesenchymal stromal cells (MSC) up-regulate podoplanin with unknown consequences for disease pathogenesis. The function of podoplanin has been linked to enhanced migratory potential and interactions with platelets. However, it is unclear how these two cell types interact with one another, given that MSC and platelets are usually located in different anatomical compartments (tissue vs blood respectively) separated by the blood vascular endothelial cells (EC).

Objectives Here, we examined the functional consequences of podoplanin expression on the migratory potential of MSC and their interactions with circulating platelets.