patients, however their ligands, CXCL8 and CXCL6, were lost from the territorial matrix of chondrocytes. Blockade of CXCR1/2 signalling at receptor level in chondrocytes resulted in a significantly reduced extracellular matrix sulphated glycosaminoglycan content of micromass cultured chondrocytes and a significantly reduced expression of the chondrocyte differentiation markers COL2A1, Aggrecan and SOX9. CXCR2 and CXCL5 were expressed in unchallenged wild type mouse articular cartilage. $CXCR2^{-/-}$ mice subjected to the DMM model and analysed 8 weeks following surgery developed a significantly more severe osteoarthritis phenotype than wild type controls.

Conclusions Our findings indicate that CXCR1/2 signalling is required for the maintenance of phenotypic stability of articular chondrocytes. We show that mouse CXCR2 signalling is required for articular cartilage homeostasis and is chondroprotective during conditions of challenge in vivo.

A8.18 VISFATIN/PBEF IN BONE REMODELLING OF RHEUMATOID ARTHRITIS

doi:10.1136/annrheumdis-2013-203222.18

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Objectives Rheumatoid arthritis (RA) is associated with increased production of the adipocytokine visfatin in synovial fluid and tissue of RA patients. Visfatin promotes the synthesis of pro-inflammatory and matrix-degrading effector molecules in RA synovial fibroblasts. Moreover, an immunohistochemical analysis of RA bone tissue showed a co-localisation of visfatin with key cells of bone remodelling (osteoblasts, osteoclasts) but the role of this adipokine in processes of bone remodelling in RA is unclear. In this study, we focussed on visfatin and its influence on RA osteoblast and osteoclast activity and differentiation as well as on its immunomodulatory properties.

Methods Human osteoblasts and osteoclasts were isolated from bone tissue and blood samples of RA patients and stimulated with visfatin. Visfatin-mediated effects on osteoblasts and osteoclasts were analysed on the transcriptional and translational level using realtime polymerase chain reaction and immunoassays. Additionally, effects of visfatin on matrix-production of osteoblasts as well as differentiation and resorption activity of osteoclasts were examined by Alizarin-Red S-, TRAP- and von Kossa-staining.

Results Stimulation with visfatin induced the secretion of pro-inflammatory cytokines (*e.g.* IL-6: 5-fold increase; IL-8: up to 100-fold) in RA osteoblasts. Additionally, quantitative realtime PCR showed several genes being differentially expressed in osteoblasts after stimulation with visfatin (*e.g.* alkaline phosphatase, OPG, Osterix). In contrast, osteoclasts only weakly respond to visfatin. A regulation on translational level was observed with regard to the production of the cytokines IL-6 and IL-8, showing a moderate increase.

Conclusions The results of the present study indicate that visfatin influences the activity as well as the differentiation of human osteoblasts in RA by modulating the expression of genes being involved in matrix production and osteoblast phenotype development. These results support the idea of visfatin affecting bone metabolism in RA. Furthermore, the finding of cytokine-induction in osteoblasts and osteoclasts in RA confirm the pro-inflammatory potential of visfatin in RA.

Acknowledgement Funded by the German Research Society (SPP1468, IMMUNOBONE, NE1174/6–1).

9. Novel therapeutic targets

A9.1 AVB3 INTEGRIN INHIBITION WITH CILENGITIDE BOTH PREVENTS AND TREATS COLLAGEN INDUCED ARTHRITIS

doi:10.1136/annrheumdis-2013-203223.1

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Background Rheumatoid arthritis (RA) is characterised by synovial inflammation and osteoclast (OC) mediated bone erosions. AlphaVbeta3 (αvβ3) integrin is highly expressed in OCs. Avβ3 blocking antibodies reduce bone resorption and mice lacking β3 are osteopetrotic.

Objectives Efficacy testing of the αvβ3 inhibitor cilengitide, a synthetic Arginine-Glycine-Asparagine amino acid peptide (RGD peptide), on osteoclastogenesis and the collagen induced arthritis (CIA) model for human RA.

Materials and Methods In vitro mouse bone marrow-derived cells (BMCs) were differentiated into tartrate resistant acid phosphatase positive (TRAP+) mononuclear OC precursor cells (pre-OCs) and TRAP+ multinucleated mature OCs with macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappaB ligand (RANKL). Cilengitide, was added in increasing concentrations (2 nM to 20 μM) to the culture. These osteoclastogenesis assays were performed on plates coated with RGD containing matrixes osteopontin, fibronectin and fibrinogen but also on Poly-D-lysine to assess αvβ3 independent adhesion. In vivo CIA was induced in 6–8 week old male DBA/1 mice by immunisation with bovine type II collagen at day 1, followed by boosting at day 21. For CIA prevention mice received subcutaneously (s.c.) 1.5 mg/kg cilengitide (n = 15) or placebo (n = 15), 5 days per week, 1 day prior to CIA induction until day 53. For CIA treatment, mice with arthritis were randomised and received 1.5 mg/kg (low dose, n = 19) or 75 mg/kg (high dose, n = 7) cilengitide or placebo (n = 21), until day 59. Preventive and treatment effects were evaluated by assessing paw thickness and grip strength.

Results In vitro increasing concentrations of cilengitide (IC50: 250 nM) dose-dependently reduced pre-OCs on all coatings, indicating early inhibition at the pre-OC proliferation stage. OCs were reduced above 200 nM, followed by complete disappearance above 2 μM. At 200 nM an intriguing morphological difference with reduction in OC size suggested that cilengitide may disrupt spreading and fusion capacity at the early pre-OC stage. In vivo CIA prevention with cilengitide effectively reduced incidence (92.8% versus 40%) and severity of arthritis as evidenced by reduction of clinical disease activity scores. Low and high dose cilengitide effectively inhibited progression of established arthritis.

Conclusions Osteoclastogenesis requires intact αvβ3 integrin function. Systemic αvβ3 integrin inhibition with cilengitide potently prevents and treats experimental CIA. Cilengitide may be a novel therapeutic target in RA.

A9.2 THE PHOSPHOINOSITIDE 3-KINASE PATHWAY REGULATES FIBROBLAST-LIKE SYNOVIOCYTES INVASION

doi:10.1136/annrheumdis-2013-203223.2

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Background/Objectives Cartilage destruction mediated by invasive fibroblast-like synoviocytes (FLS) plays a central role in pathogenesis of RA. Increased cell migration and degradation of extracellular matrix are fundamental to these processes. The Class I phosphoinositide 3-kinases (PI3K) control cell survival, proliferation and migration, which might be involved with cartilage damage in