disease of the joints. Whilst the autoimmune element continues to be studied intensely, it is evident that the innate inflammatory response propagates the disease. Fibroblast-like synoviocytes (FLS) are the most abundant stromal cell in the synovium. FLS from RA joints are markedly different from their healthy counterparts in their inflammatory characteristics.

RA FLS exhibit a form of inflammatory memory named priming. Prolonged exposure to TNFα induces an augmented chemokine response to challenges with interferons [1]. Fibroblast priming leads to significantly augmented IL6, CCL5 and CXCL10 responses to a second challenge by TNFα, with a coincident prolonged NFκB nuclear localization (manuscript in press).

Objectives: We wished to assess whether fibroblasts from sites other than the synovial joint would also exhibit this primed response to second challenge.

Methods: Fibroblasts from the joint, skin, lung, tonsil, bone marrow and gum were stimulated with 10ng/ml TNFα for 24h, before the conditioned medium was removed and the cells were washed free of stimulus. Cells were rested for 24h before once again being washed and stimulated with 10ng/ml TNFα for a further 24h. The conditioned medium was removed and secreted mediators compared between first and second response to stimulus. Transcription and intracellular signalling at time points within each challenge were also determined.

Results: Synovial, lung and tonsil fibroblasts augmented IL6 secretion in response to the second TNFα challenge. RA bone marrow-derived fibroblasts varied in their exhibition of priming. Skin fibroblasts from patients undergoing cosmetic surgery and a gingival sample from non-chronic inflammation displayed no evidence of IL6 priming.

Fibroblasts from the skin of psoriasis (Ps) patients mounted a primed response that was significantly augmented compared to their first response to TNFα, and significantly higher than the second response of healthy skin. This augmented response matches that of FLS, as IL6 but not IL8 was increased. This pattern was also matched by gum fibroblasts from periodontitis.

Mechanistically the Ps skin fibroblasts match FLS, in that NFκB nuclear localization is prolonged in the second challenge compared to the first.

Conclusions: We have shown that inflammatory memory in the form of IL6 priming occurs in fibroblasts from a variety of anatomical sites with diverse functions. Its prevalence implies a shared phenomenon, but the variation between sites suggests a specific role required in some tissues and not others.

The finding that Ps skin fibroblasts acquire this memory response may point towards a pro inflammatory mechanism that contributes to psoriatic diseases, including psoriatic arthritis. Our data may help to explain why an estimated 30% of psoriasis patients go on to develop psoriatic arthritis [2].

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OP0179 BRAFV600E PROMOTES MYELOID SKEWING IN MULTISYSTEMIC LANGERHANS CELL HISTIOCYTOSIS HUMANIZED MOUSE MODEL

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Background: Multisystemic Langerhans cell histiocytosis (mLCH) is an aggressive disease characterized by the accumulation of mononuclear phagocytes with immunohistochemical features of dendritic cell (histiocytes)[1]. Histiocytes infiltrate mostly the skin, bone marrow (BM), lung and spleen, and produce high levels of proinflammatory cyto/chemokines, leading to organ dysfunction[2,3]. In patients, around 10% of cells in lesions carries an oncogenic mutation in the MAPK pathway, mostly $BRAF^{V600E}$ (70% of cases). $BRAF^{V600E}$ can be detected also in monocytes and BM progenitors (HSPC) from these patients, whereas only a fraction of them carries a mutation in B cells and none in T cells[4].

Objectives: To study the role of $BRAF^{V600E}$ in the pathogenesis of mLCH, we set up a humanized mouse model of mLCH based on the transplantation into immunodeficient mice (NSG) of human HSPC expressing $BRAF^{V600E}$.

Methods: We isolated HSPC from human cord blood and transduced them at two different levels (50% and 20%) with lentiviral vectors that ubiquitously express $BRAF^{V600E}$, $BRAF^{WT}$ or GFP.

Results: All $BRAF^{V600E}$ mice manifested severe weight loss within 7 weeks with a median of 3 and 5 weeks for 50% and 20% transduction groups, respectively. Mice showed dysplastic bone marrow (BM) with infiltration of histiocytes; lesions were present also in lungs, kidneys, CNS, spleen and liver. Immunophenotype of infiltrating histiocytes closely resembles mLCH, staining positive for CD14, CD68, S-100 and langerin. None of the control mice lost weight nor displayed organ alteration. Flow cytometry analyses of BM showed 5- to 7-fold reduction in engraftment of human cells in $BRAF^{V600E}$ vs GFP groups ($p<0,001$). Percentage of myeloid cells increased by 3- to 4-fold in $BRAF^{V600E}$ vs GFP groups ($p<0,001$). On the contrary, percentage of B cells was reduced by 3- to 6,5-fold in $BRAF^{V600E}$ vs GFP groups ($p<0,001$). Moreover, there was no difference in the percentage of GFP-positive cells between myeloid cells, whole BM and *in vitro* sample, suggesting a non-cell autonomous mechanism underlying this myeloid phenotype.

Conclusions: In summary, we generated for the first time a human xenogeneic transplantation mouse model of mLCH, showing that $BRAF^{V600E}$ in human HSPC promotes myeloid skewing rather than proliferation.

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Joint health & joint damage: a tale of three tissues –

OP0180 PHARMACOLOGICAL CHARACTERIZATION OF THE ADAMTS-5 INHIBITOR GLPG1972: AN ORAL ANTI-CATABOLIC AGENT FOR THE TREATMENT OF OSTEOARTHRITIS

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Background: Degradation of articular cartilage and alterations of the underlying subchondral bone are hallmarks of osteoarthritis (OA)[1]. A disintegrin and metalloproteinase with thrombospondin motifs-5 (ADAMTS-5) is a key aggrecan-cleaving enzyme involved in this pathogenic process from the earliest stages of cartilage degradation[2] and as such, is an attractive drug target for the development of a disease-modifying OA drug (DMOAD)[3].

Objectives: In this report we describe the *in vitro* and *in vivo* characterization of the small molecule GLPG1972, an inhibitor of ADAMTS-5. GLPG1972 anti-catabolic activity was evaluated in murine and human cartilage explants and DMOAD activity was investigated in the destabilization of the medial meniscus (DMM) mouse model[4].

Methods: The ADAMTS-5 biochemical assay is based on the cleavage of a fluorescent substrate by recombinant ADAMTS-5. Mouse femoral head cartilage explants were stimulated by interleukin-1a (IL-1a) for 3 days and GAG release quantified[2b]. Human articular cartilage explants were stimulated with IL-1β for 12 or 19 days and the NITEGE epitope quantified using the AGNx1 assay. Unilateral OA was induced in C57BL6 mice by DMM[4]. Mice were treated with vehicle or GLPG1972 at 30, 60 or 120 mg/kg, b.i.d. for 8 weeks. Medial femorotibial joint sections were scored by an evaluator blinded to treatment.

Results: GLPG1972 showed potent inhibition of human ADAMTS-5 (IC_{50}=20 nM). Inhibition of ADAMTS-4 was moderate (IC_{50}=57 nM), and selectivity over 100-fold was observed against a large panel of zinc metalloproteinases. GLPG1972 displayed potent anti-catabolic activity in cartilage explants, with IC_{50} values being 2 μM and <1 μM in mouse and human, respectively. In the DMM mouse model, GLPG1972 demonstrated DMOAD activity, as shown by significant reduction of femorotibial cartilage proteoglycan loss and cartilage damage score, as well as significant impact on subchondral bone sclerosis.

Conclusions: GLPG1972 is an orally bioavailable, potent and selective ADAMTS-5 inhibitor showing significant anti-catabolic activity in cartilage explants. In the DMM model, treatment with GLPG1972 resulted in significant protective effects on both cartilage and subchondral bone pathology. Taken together these results provide support to progress GLPG1972 into the clinic as an oral treatment for OA.

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OP0181 DELETION OF THE PROSTAGLANDIN D2 RECEPTOR DP1 EXACERBATES AGING-ASSOCIATED AND INSTABILITY-INDUCED OSTEOARTHRITIS

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Background: *The D prostanoid receptor 1* (D*P1*), a *receptor* for prostaglandin D_2 (PGD_2) plays important roles in inflammation and cartilage metabolism. However, its role in the pathogenesis of osteoarthritis (OA) remains unknown.

Objectives: We undertook this study to explore the roles of DP1 in the development of OA and to evaluate the efficacy of a DP1 selective agonist in the treatment of OA.
Methods: We compared the development of aging-associated OA and destabilization of the medial meniscus (DMM)-induced OA in DP1-deficient (DP1-/-) and wild-type (WT) mice. The progression of OA was assessed by histology, immunohistochemistry, and microcomputed tomography (micro-CT). Cartilage explants from DP1-/- and WT mice were treated with interleukin-1α (IL-1α) ex vivo, to evaluate proteoglycan degradation. The effect of intra-peritoneal administration of the DP1 selective agonist BW245C on OA progression was evaluated in WT mice.
Results: Compared to WT mice, DP1-/- mice had exacerbated cartilage degradation in both models of OA and this was associated with increased expression of MMP-13, and ADAMTS-5. In addition, DP1-/- mice demonstrated enhanced subchondral bone changes. Cartilage explants from DP1-/- mice showed enhanced proteoglycan degradation following treatment with IL-1α. Intraperitoneal injection of BW245C attenuated the severity of DMM-induced cartilage degradation and bony changes in WT mice.
Conclusions: These findings indicate a critical role for DP1 signaling in OA pathogenesis. Modulation of DP1 functions may constitute a potential therapeutic target for the development of novel OA treatments.
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OP0182 CONVERGENCE OF JOINT REPAIR AND PAIN PATHWAYS VIA NERVE GROWTH FACTOR AND P75 EXPRESSING MESENCHYMAL STEM CELLS- A NOVEL EXPLANATION FOR OSTEOARTHRITIS PROGRESSION WITH ANTI-NGF IN OSTEOARTHRITIS

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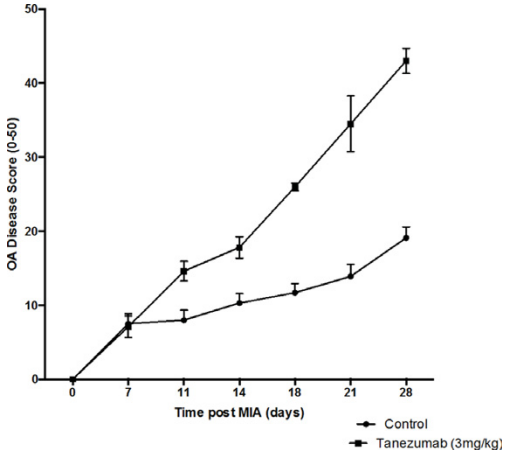
Background: Nerve growth factor (NGF) is a key regulator of pain and anti-NGF therapy reduces osteoarthritis (OA) associated pain. However, anti-NGF therapy is associated with rapidly progressive OA (RPOA) [1]. In hip OA there is a 5-fold increase in mesenchymal stem cells (MSCs) from MRI bone marrow (BM) lesions, areas associated with OA progression [2]. MSCs in such lesions are uniformly positive for the NGF receptor, p75, which is also linked to chemotaxis and proliferation in other stromal cell compartments [3].
Objectives: Evaluate anti-NGF treatment in monoiodoacetate (MIA) induced OA and test whether NGF influences human BM-MSC function.
Methods: Human tibial plateau (TP) bone was isolated from patients undergoing total knee replacement. OA was induced in male Wistar rats (n=6) by intra-articular injection of 0.3 mg MIA. Each animal was treated with subcutaneous injection of control human IgG or anti-NGF (3 mg/kg) at Days 5, 10 and 15. Human and animal tissues sections were prepared for histological analysis using H&E staining and immunohistochemistry (IHC) using anti-p75 and anti-NGF antibodies. BM-MSCs were isolated from iliac crest aspirates and cultured under normal conditions. Expression of p75 was induced following overnight incubation with 400mM ethanol (EtOH) and confirmed by flow cytometry. Proliferation was assessed ± EtOH and 0–1 μg/ml NGF.
Results: Regions adjacent to cartilage loss in human TP showed abundant stromal proliferation, NGF and p75 immunoreactivity. In MIA model, by Day 18 there was substantial loss of cartilage, bone remodelling and stromal proliferation mimicking human disease. By Week 4 animals demonstrated unequal weight-bearing ($p<0.05$). Anti-NGF provided sufficient analgesia to normalise weight-bearing. Compared to IgG control, arthropathy progression was faster (Fig) with complete cartilage loss, bone marrow necrosis and cyst formation by Day 21. p75 immunoreactivity was greatly reduced in MIA-injected rats receiving anti-NGF compared with IgG. NGF positive staining was widespread in naïve and MIA-injected knees, but almost absent from MIA-treated knees of anti-NGF treated rats.



To investigate increased p75 positivity in knee OA, we restored p75 expression loss in expanded cell, to 97% of BM-MSCs (n=3) using EtOH. Increased MSC proliferation was seen at Days 6 and 9 (26%, p=0.03 and 30%, p=0.01 increase respectively, n=7) for 1 μg/ml NGF in the presence of EtOH compared to control (no NGF). In cultures without EtOH induction (absent p75) NGF had no effect.
Conclusions: TP bone from OA patients and rat MIA-treated subchondral bone contains p75 positive staining in regions of cartilage destruction and associated NGF positivity. Anti-NGF treatment exacerbated MIA-induced OA and reduced NGF and p75 expression. In vitro, NGF increased BM-MSC proliferation, suggesting NGF may be involved in the stromal proliferation seen at sites of OA damage. Thus, NGF may regulate MSC function. Complete blockade represents a novel mechanism for accelerated joint destruction in OA.
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OP0183 CORDYCEPIN, A NOVEL COMPOUND, REDUCES KNEE JOINT PATHOLOGY AND PAIN IN THE MONOSODIUM IODOACETATE (MIA) RAT MODEL OF OSTEOARTHRITIS

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Background: Cordycepin (3' deoxyadenosine) is a popular traditional medicine in Asia, taken for conditions associated with ageing. Osteoarthritis (OA) is a common cause of pain and disability in the ageing population. Inflammation is a key component of osteoarthritic pain. Cordycepin is thought to act by inhibiting polyadenylation, the last step of mRNA synthesis, and can potentially have increased therapeutic benefit in OA (anti-inflammatory and analgesic) with fewer side effects than currently available therapies.
Objectives: The aim of this study was to determine whether cordycepin treatment alters osteoarthritic pain and pathology, and to decipher the mechanisms of action by which cordycepin exerts any potential beneficial actions.
Methods: OA was induced in male Sprague Dawley rats by intra-articular injection of mono-sodium iodoacetate (MIA; 1mg/50μl) on day 0. Cordycepin was administered orally (2mg/rat mixed in 1g of wet mash) every other day for 2 weeks (pre-emptive study: day 0 to day 14, therapeutic study: day 14 to day 28). Pain behaviour was measured as hind-limb weight-bearing asymmetry and mechanical paw withdrawal thresholds. Joint tissues were collected at days 14 and 28. Joint changes were quantified using histology and immunohistochemistry techniques. Synovial inflammation was quantified as extent of CD68 positive macrophage and cellular infiltration. Synovial angiogenesis was measured as endothelial cells positive for proliferating cell nuclear antigen (PCNA). Safranin-O staining was used to score cartilage damage and bone changes (osteophytes and channels crossing the osteochondral junction [OCJ]). Tartrate-resistant acid phosphatase (TRAP) positive osteoclasts and ADAMTS-5 and MMP13 positive chondrocytes were quantified as additional markers to detect bone and cartilage changes.
Results: The MIA rodent model of OA pain exhibited significant pain behaviour, synovial inflammation and angiogenesis, cartilage damage, osteophyte formation and subchondral bone changes, compared with non-arthritic controls. A two week pre-emptive and therapeutic treatment with cordycepin reduced MIA-induced pain behaviour and synovial changes (inflammation and angiogenesis). Pre-emptive cordycepin treatment reduced cartilage damage and the level of ADAMTS-5 and MMP13 from the chondrocytes. Pre-emptive and therapeutic cordycepin treatment reduced the number of channels crossing the OCJ and TRAP positive osteoclasts in the subchondral bone, but had no effect on numbers of osteophytes. Therapeutic cordycepin treatment did not alter cartilage damage score or the level of ADAMTS-5 and MMP13 positive chondrocytes.
Conclusions: Our data show that the analgesic effects of orally administered cordycepin in a pre-emptive and therapeutic protocol are associated with synovial changes (inflammation and angiogenesis) and bone remodelling. Administration of cordycepin before the onset of MIA-induced OA reduced cartilage damage and had a chondroprotective effect. Whereas therapeutically administered cordycepin did not alter cartilage damage. Further studies will investigate whether cordycepin mediated reduction in MIA-induced pathology and pain behaviour is as a result of its direct action on polyadenylation inhibition. Polyadenylation inhibitors could therefore be a novel class of drugs for treating OA.
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