2. Haywood KL, Garratt AM, Dziedzic K, Dawes PT. Patient centered assessment of ankylosing spondylitis-specific health related quality of life: evaluation of the Patient Generated Index. J Rheumatol. 2003; 30: 764–73.

OP24. THE EFFECT OF SOCIAL DEPRIVATION IN ANKYLOSING SPONDYLITIS

Emma L. Healey[1,2], Kirstie L. Haywood[3,1], Kelvin P. Jordan[1], Andrew M. Garratt[4] and Jon C. Packham[2,1]
[1]*Arthritis Research Campaign National Primary Care Centre, Keele University, Newcastle-under-Lyme, United Kingdom,* [2]*Staffordshire Rheumatology Centre, Haywood Hospital, Stoke-on-Trent, United Kingdom,* [3]*RCN Research Institute, School of Health & Social Studies, Warwick University, Coventry, United Kingdom and* [4]*National Resource Centre for Rehabilitation in Rheumatology, Diakonhjemmet Hospital, Oslo, Norway*

Background: The strong correlation between social deprivation and health is well described. For example, rheumatoid arthritis patients with lower socioeconomic status have been shown to have more severe disease, more comorbidity and higher mortality. However, little is known about the impact of area-level social deprivation on the health of Ankylosing Spondylitis (AS) patients. The aim of this study was to determine the association of disease severity in AS with the extent of deprivation in the local area.
Methods: 800 AS patients from 8 specialist rheumatology centres across England were invited to participate in a cross-sectional survey. Socio-demographic & disease related variables (pain [NRS], function [BASFI] and disease activity [BASDAI]) were collected on a self-reported questionnaire. Deprivation was measured using the Index of Multiple Deprivation (IMD) 2004. Scores are allocated to areas with a mean population of 1500 based on seven specific features of local neighbourhoods in England (i.e. Employment, Income, Housing, Environment, Health, Crime, Education). A weighted overall deprivation score is determined based on these domains. Patients were allocated to an area by postcode.
Results: 468 patients entered the study (response rate 58.5%, range per centre 51.7–70.6%). 72.9% were male, mean age 50.2 years (s.d. 12.1), mean diagnosed disease duration 17 years (s.d. 11.4). Patients were divided based on deprivation domain quintile scores, with 93 patients in the least and most deprived categories, & the middle three quintiles combined into a mid group ($n = 282$). There was a good spread of deprivation levels between the centres, with Cambridge and Bristol being least deprived and Stoke and Newcastle being most deprived. Although there was a trend towards more severe disease in the more geographically deprived centres, this did not reach statistical significance. However, across all centres, those living in more deprived areas generally demonstrated corresponding worse disease (disease activity, pain and function) & were more likely to be older, single males. After controlling for age, gender and marital status, separate logistic regression analyses revealed that greater overall deprivation was significantly associated with greater pain (OR 1.52; CI 1.12, 2.06), greater disease activity (OR 1.93; CI 1.42, 2.63) & poorer function (OR 2.03; CI 1.48, 2.78).
Conclusions: This study suggests that need for healthcare is greatest amongst those from more deprived areas. However, it has been shown that this group are the least likely to access healthcare and receive interventions. With the growing use of anti-TNF therapies, this has important implications if health service resources are to be allocated equitably.
Disclosure: J.C.P. received an unrestricted educational research grant from Wyeth. All the other authors have declared no conflicts of interest.

Concurrent Oral 5 – Aetiopathogenesis of Rheumatic Disease

OP25. FUNCTIONAL ROLE OF DR3 IN INFLAMMATORY ARTHRITIS: A NOVEL TARGET FOR THERAPY?

Anwen S. Williams[1], Melanie J. Bull[2], Zarabeth Mecklenburgh[2], Claudia J. Calder[2], Jason P. Twohig[2], Carole Elford[3], Bronwen Evans[3], Aymen Al-Shamkhani[4] and Eddie Wang[2]
[1]*Rheumatology, Cardiff University, Cardiff, United Kingdom,* [2]*Medical Biochemistry and Immunology, Cardiff University, Cardiff, United Kingdom,* [3]*Child Health, Cardiff University, Cardiff, United Kingdom and* [4]*Cancer Science Division, University of Southampton, Southampton, United Kingdom*

Background: Death receptor 3 (DR3) is a death domain containing member of the tumour necrosis factor receptor superfamily (TNFRSF). DR3 signals as a receptor trimer, recruiting intracellular signaling molecules in order to bring about the induction of apoptosis. It is also capable of promoting cell survival through the activation of NFkB. Currently TNF-like protein 1A (TL1A)is the only confirmed ligand for DR3. Published genetic data implicate DR3 in rheumatoid arthritis (RA). We provide in vitro and in vivo functional data to test the hypothesis that the DR3/TL1A pathway is important in the pathogenesis of inflammatory arthritis.
Methods: We used our colony of DR3 deficient (DR3ko) mice and littermate wild-types (DR3wt) as controls. Arthritis progression was assessed in the murine antigen-induced arthritis (mAIA) model. The therapeutic effect of antagonising the DR3 pathway was measured in the murine collagen-induced arthritis (mCIA) model using a novel anti-TL1A monoclonal antibody. Histological disease activity indices were compared in anti-TL1A versus IgG2A treated mice. Osteoclast numbers were quantified in vivo and in vitro by tartrate resistant acid phosphatase staining.
Results: mAIA resolution was significantly quicker in DR3ko than DR3wt. All disease activity indices were significantly reduced in DR3ko over DR3wt. We found that DR3ko were completely protected from the development of the focal bone erosions characteristically seen in this model during the chronic phase. In addback experiments we demonstrated that these effects on mAIA were DR3 specific. We found that osteoclast numbers were significantly elevated at the bone/pannus interface in DR3wt over DR3ko and that activation of the DR3 pathway with TL1A caused heightened osteoclast formation in DR3wt bone marrow macrophages but not in DR3ko cells. Significantly heightened osteoclast formation was also observaed in human peripheral blood mononuclear cell cultures containing TL1A over controls differentiated in the absence of TL1A. When we tested the therapeutic efficacy of DR3/TL1A antagonism with an anti-TL1A antibody we found that the anti-TL1A reduced the incidence and severity of mCIA. Indeed arthritis was virtually ameliorated at endpoint.
Conclusions: Our results indicate an important role for DR3 in two experimental models of inflammatory arthritis, most particularly with respect to the development of adverse bone pathology. Our proof of concept data suggest that DR3/TL1A antagonism could be a valid target for therapy in inflammatory arthritides and diseases characterised by adverse bone pathology.
Acknowledgements: We also acknowledge and thank Dr Tania F. Rowley, Dr Tomasz J. Slebioda and Dr Vadim Y. Taraban (Cancer Sciences Division, University of Southampton School of Medicine)for their valuable contributions in generating, purifying and characterising the anti-TL1A reagent used in this study.
Disclosure: The authors have declared no conflicts of interest.

OP26. DOES LIGHT PLAY A ROLE IN THE PATHOGENESIS OF INFLAMMATORY JOINT DESTRUCTION?

Catherine Swales, David Mahoney, Paul Wordsworth, Nick Athanasou and Afsie Sabokbar
Nuffield Department of Orthopaedic Surgery, Botnar Research Centre, University of Oxford, Oxford, United Kingdom

Background: Osteoclasts are responsible for the localised and systemic bone loss that characterise rheumatoid arthritis (RA). Osteoclast differentiation from monocyte precursors requires macrophage colony-stimulating factor (M-CSF) and receptor activator for nuclear κB ligand (RANKL); the decoy receptor osteoprotegerin (OPG) blocks RANKL-mediated osteoclast formation. Osteoclastogenesis is also modulated by tumour necrosis factor (TNF) and members of its superfamily such as LIGHT [homologous to Lymphotoxins exhibiting Inducible expression and competing with herpes simplex virus Glycoprotein D for Herpesvirus entry mediator, a receptor expressed by T lymphocytes]. Recent evidence suggests that LIGHT is over-expressed in the serum of RA patients and promotes RANKL-dependent and -independent osteoclastogenesis from monocyte precursors. LIGHT's action may be modulated by the non-signalling decoy receptor 3 (DcR3). This study investigates the role of LIGHT in osteoclastogenesis from synovial fluid macrophages of patients with inflammatory joint disease.
Methods: Synovial fluid (SF) was aspirated from knee joints of patients with clearly identifiable diagnoses on the basis of clinical, radiological and histological criteria (RA, gout, pyrophosphate arthropathy, inflammatory and non-inflammatory osteoarthritis (OA)). SF samples from non-inflammatory OA represented the "control" group. Isolated SF macrophages were cultured on dentine slices in the presence of M-CSF (survival factor of osteoclast precursors) $\pm$ RANKL (positive control) and modulators of osteoclastogenesis - LIGHT, TNFα, OPG and DcR3. Evidence of osteoclast formation was assessed by staining for tartrate-resistant acid phosphatase, a cytochemical marker of osteoclasts; functional activity was determined by the percentage area of resorption on dentine slices. SF levels of LIGHT, DcR3 and TNFα were determined by ELISA.
Results: This study shows that:

1) Synovial fluid levels of LIGHT are significantly greater than TNFα in all arthritis patients, regardless of underlying diagnosis
2) Synovial fluid levels of DcR3 (and therefore modulation of LIGHT) varies significantly between different inflammatory arthropathies
3) LIGHT not only augments RANKL-dependent osteoclastogenesis but also induces RANKL-independent osteoclast formation from SF macrophages
4) TNFα reduces LIGHT-mediated osteoclast formation and lacunar resorption.

Conclusions: Our results demonstrate for the first time that LIGHT is an important regulator of osteoclastogenesis from synovial fluid macrophages of patients with inflammatory joint disease. In addition, the ratios of LIGHT:DcR3 and LIGHT:TNFα in synovial fluid are crucial determinants of the extent of RANKL-independent bone resorption. As LIGHT is found in greater concentrations than TNFα in all types of synovial fluid, it may have a profound impact on the pathogenesis of inflammatory and erosive joint disease and therefore represents a potential therapeutic target.
Disclosure: The authors have declared no conflicts of interest.

OP27. LOCALLY GENERATED GLUCOCORTICOIDS, RATHER THAN PRO-INFLAMMATORY CYTOKINES, DIRECTLY REGULATE SYNOVIAL DKK-1 EXPRESSION IN INFLAMMATORY ARTHRITIS

Rowan S. Hardy[1], Mohammad Ahasan[1], Pushpa Patel[1], Elizabeth Rabbitt[1], Andrew Filer[2], Karim Raza[2], Paul Stewart[1], Chris Buckley[2] and Mark S. Cooper[1]

[1]School of Clinical and Experimental Medicine, The University of Birmingham, Birmingham, United Kingdom; [2]Immunity and Infection, The University of Birmingham, Birmingham, United Kingdom

Background: We have previously proposed a central role for locally generated glucocorticoids in the periarticular and systemic osteoporosis seen in rheumatoid arthritis (RA). Fibroblasts like synoviocytes (FLS), which form a substantial component of the inflamed synovium, possess high expression of the glucocorticoid generating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). This expression is significantly increased in response to pro-inflammatory cytokines. Recently, production of DKK-1 (a Wnt signalling inhibitor known to inhibit bone formation and support bone resorption) by FLS in response to inflammation has been proposed to be a master regulator of inflammatory bone loss. We tested the hypothesis that DKK-1 production in inflamed synovium was primarily mediated by glucocorticoids and indirectly by inflammatory cytokines.
Methods: Primary FLS were isolated from synovial biopsies from 4 patients with RA and 3 with osteoarthritis (OA) undergoing orthopaedic surgery. Glucocorticoid metabolism by 11β-HSD1 was measured in assays using tritiated tracers and thin layer chromatography. DKK-1 expression was measured by real-time RT-PCR and ELISA on cell culture supernatant. Regulation of the DKK-1 promoter was evaluated using luciferase assay.
Results: High basal DKK-1 mRNA and protein expression were found in FLS. In the RA group TNFα treatment resulted in a small increase in expression (2.3 fold in mRNA; 1.4 fold in protein) whereas IL-1 had no effect. The active glucocorticoids cortisol and dexamethasone caused a substantially greater increase in mRNA and protein expression (3.1 and 3.2 fold increase in mRNA, $p<0.05$; 2.3 and 2.8 fold in protein respectively, $p<0.05$). Importantly, the inactive glucocorticoid cortisone also increased DKK-1 expression in FLS (2.7 fold in mRNA, $p<0.05$; and 1.7 fold in protein, $p<0.05$) to a much greater degree than TNFα/IL1, an effect blocked by an 11β-HSD1 inhibitor. Similar changes were seen in DKK-1 promoter reporter assays. When glucocorticoids and TNFα/IL1 were combined the effect on DKK-1 expression was similar to that of glucocorticoids alone. Even though the direct effects of TNFα/IL-1 on DKK1 were modest/absent both cytokines were able to substantially increase 11β-HSD1 expression and glucocorticoid production in these cells. Comparable basal expressions of DKK-1 were observed in FLS from patients with OA, with an identical response to glucocorticoids and inflammatory cytokines.
Conclusions: These results show that the effect of TNFα/IL-1 on FLS DKK-1 expression, and any consequences on bone remodelling, are unlikely to be direct but instead are mediated indirectly through increased local glucocorticoid generation.
Disclosure: The authors have declared no conflicts of interest.

OP28. INTRACELLULAR SIGNALLING IN RESPONSE TO WOUNDING OF CONNECTIVE TISSUES

Fiona Watt, Athanasios Didangelos, Matthew Peirce, Robin Wait and Jeremy Saklatvala
Kennedy Institute of Rheumatology, Imperial College London, London, United Kingdom

Background: Injury to a joint predisposes to osteoarthritis. The response of connective tissues to injury, and how this leads to degeneration is not understood. Our group has previously shown that the mitogen activated protein kinases (MAPKs), typically activated by inflammatory stimuli such as IL-1 and lipopolysaccharide are also activated by explantation (sharp dissection) of cartilage. I set out to examine a) whether other intracellular signalling pathways such as NFκB and tyrosine kinases were activated by cartilage injury, and b) whether other connective tissues such as synovium responded in a similar manner.
Methods: Porcine cartilage or synovial lysates were assayed for phospho-kinases by western blotting. IκB kinase (IKK) activity was assayed by immunoprecipitation-kinase assay, by measuring phosphorylation of the substrate IκBα. Phosphotyrosine western blotting or immunoprecipitation was carried out with 4G10 antibody. Phosphorylated proteins were eluted from immunoprecipitates with phenyl phosphate, electrophoresed, and bands silver stained and identified by tandem mass spectrometry.
Results: A number of intracellular signalling pathways were activated following injury of either cartilage or synovium. These included MAPKs, IKK, tyrosine kinases and PI3 kinase. The activation was evident within 30 seconds of injury and was not dependent on exposure to culture medium. The same pattern of tyrosine phosphorylation was induced by injury to cartilage or synovium. These conserved phospho-proteins were identified by mass spectrometry as focal adhesion kinase (FAK), paxillin (a FAK substrate) and cortactin (a src substrate).
Conclusions: Connective tissues respond to injury via an unknown mechanism. The signalling response is rapid and conserved between tissues. FAK/src may represent mechano-transducing kinases in connective tissues. IKK activation, which leads to induction of NFκB, is likely to be catabolic for the extracellular matrix, and may represent a mechanism whereby injury gives rise to degradation.
Disclosure: The authors have declared no conflicts of interest.

OP29. ANALYSIS OF DISEASED POSTERIOR TIBIALIS TENDON SPECIMENS REVEALS COMMON MOLECULAR PATHOGENESIS OF TENDINOPATHY AT DIFFERENT ANATOMICAL SITES

Graham Riley[1,4], Anthony Corps[4], Nicholas Avery[3], Valerie Curry[4], Andrew Robinson[2], Rebecca Harrall[4] and Brian Hazleman[4]
[1]School of Biological Sciences, University of East Anglia, Norwich, United Kingdom, [2]Department of Trauma and Orthopaedics, Addenbrooke's Hospital, Cambridge, United Kingdom, [3]Matrix Biology Research Group, University of Bristol, Bristol, United Kingdom and [4]Rheumatology Research Unit, Addenbrooke's Hospital, Cambridge, United Kingdom

Background: Pain and dysfunction of the human posterior tibialis tendon (PTT) is associated with adult acquired flat foot problems, particularly in women of middle age. Little is known about the aetiopathogenesis of this condition. In this study, we collected PTT and FDLT taken at surgery, and investigated biochemical and molecular markers of matrix turnover, including the expression of mRNA encoding a range of tendon matrix proteins and metalloproteinases.
Methods: Tendon specimens were obtained from tissue discarded during surgery for the repair of PTT using FDLT transfer. The principal specimen group consisted of paired samples of PTT and FDLT tissue from 25 female patients, age 30-77 years, median 60 years. Tissue glycosaminoglycan (GAG) and collagen content were analysed by standard biochemical analysis. Pentosidine and collagen cross-links were measured by HPLC. Total RNA was isolated from frozen tissue and real-time semi-quantitative RT-PCR reactions were performed using standard techniques.
Results: Diseased PTT contained more than 2-fold higher levels of GAG than normal PTT ($P<0.05$) and 6-fold higher levels than the FDLT ($P<0.001$). The total collagen content in the three different groups was not significantly different. In normal PTT there was a linear increase of pentosidine content with increasing age and similar levels were found in the paired FDLT. In contrast, diseased PTT contained lower levels of pentosidine than expected ($P<0.05$). Diseased PTT showed significantly increased mRNA expression of a number of genes (COL1A1, COL3A1, aggrecan, biglycan, MMP-2, -13 and -23 and ADAM-12) compared with normal PTT or FDLT. ADAMTS-1 mRNA was lower in diseased PTT than in the FDL tendons, while MMP-3 and ADAMTS-5 were lower in both diseased PTT and FDLT than in the normal PT.
Conclusions: The molecular changes described here are comparable with previous studies in other tendons, consistent with a similar molecular pathogenesis of tendinopathy at different anatomical sites. The altered proteoglycan content is consistent with cellular changes induced by increased compression and/or shear forces acting at the site of tendon pathology. The increase in pentosidine content with age in normal PTT and FDLT is similar to that previously reported in biceps brachii tendons, indicating that each of these tendons normally possess a remarkably stable matrix structure in which Age-related Glycation End-products (AGE) such as pentosidine can accumulate linearly. This relationship is lost in the diseased PTT, where the disease process has led to replacement of the original collagen matrix. The cause of the increased matrix turnover found in tendinopathy is the subject of continuing controversy; the cellular response to an altered mechanical environment is generally agreed to be one of the most important factors, although whether it is a response to too much or too little mechanical strain is yet to be determined.
Disclosure: G.R. is a consultant for Wyeth Laboratories. All the other authors have declared no conflicts of interest.

OP30. THE SERUM RESPONSE PROGRAMME LEADS TO CONVERGENCE OF TRANSCRIPTIONAL PROFILES IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS SYNOVIAL FIBROBLASTS

Andrew Filer[1], Philipp Antczak[2], Greg Parsonage[1], Steve Kissane[1], Holly Legault[3], Margot O'Toole[3], Karim Raza[1], Dagmar Scheel-Toellner[1], Christopher D. Buckley[1] and Francesco Falciani[2]
[1]Rheumatology Research Group, University of Birmingham, Birmingham, United Kingdom, [2]School of Biosciences, University of Birmingham, Birmingham, United Kingdom and [3]Wyeth Research, Cambridge, MA

Background: Studies of in vitro cultured synovial fibroblasts have multiple potential confounders, including the use of high concentrations of fetal calf serum to stimulate growth. Under physiological conditions fibroblasts are not exposed to serum, but during wound healing serum exposure leads to activation of a transcriptional programme leading to growth and differentiation. We tested the hypothesis that high serum concentrations in fibroblast culture will obscure differences in gene expression of cultured fibroblasts in patients with rheumatoid arthritis (RA) and osteoarthritis (OA).
Methods: Matched synovial (SFb), bone marrow (BMFb) and skin fibroblasts (DFb) were established from 12 patients satisfying ACR criteria for RA and 6 patients with OA undergoing hip or knee arthroplasty. Cells were expanded in high serum medium (HSM, 10% FCS) to passage 5, then grown in either HS or otherwise identical low serum (LSM, 0.1% FCS) media to confluence before lysis in RNA protection buffer. Affymetrix® Genechip arrays were used to assess genome-wide gene expression changes. SAM, PCA and HCA were analysed using R with base and samr packages. DAVID was used for pathway analysis.
Results: Consistent with published data, fibroblasts from different sites and diseases clustered discretely using hierarchical cluster and principle component analyses after SAM. Anatomical site was the most powerful classifier, followed by disease, then serum status. Exposure to HSM led to parallel changes in gene expression profiles of BMFb and DFb, with RA and OA disease groups remaining in distinct clusters (Table 1). However, exposure of SFb to HSM uniquely led to convergence of RA and OA expression profiles, with no differentially expressed genes reaching statistical significance. In contrast, under LSM conditions 37 genes were upregulated and 259 genes downregulated $\geqslant 2$ fold in RA vs OA SFb with involvement of MAPKinases, cell cycle and shape change/migration pathways. Furthermore, on exposure to HSM, osteoarthritis SFb markedly downregulated 29 genes which in RA SFb were unregulated and had negligible expression regardless of serum status.

Conclusions: Two important conclusions can be drawn from this work. Firstly, the regulation of genes in OA SFb by serum exposure which are not regulated in RA SFb regardless of serum status strongly suggests that in RA these genes are under the stable control of epigenetic mechanisms. Secondly, the downregulation of multiple differentiating genes on routine in vitro HSM exposure is likely to obscure true differences between OA and RA synovial fibroblasts.

Disclosure: A.F., K.R. and C.B. have received unrestricted research grant from Wyeth. All other authors have declared no conflicts of interest.

TABLE 1. Number of differentially expressed genes attaining statistical significance by SAM

	Bone marrow: OA vs RA	Skin: OA vs RA	Synovium: OA vs RA
Low serum	94	98	302
High serum	94	97	0