Mast cells jump start K/BxN serum transfer arthritis via IL-1

Arthritis Research & Therapy Volume 9 Suppl 3, 2007

6th Global Arthritis Research Network (GARN) Meeting

Zurich, Switzerland

10-13 May 2007

Published online: 19 October 2007
These abstracts are available online at http://arthritis-research.com/supplements/9/S3

Arthritis models

P1

IL-21 modulates cytokine levels in murine collagen-induced arthritis and contributes to disease pathology

DA Young, M Hegen, H Ma, L Napierata, J Lamothe, M Senices, L Lowe, M Collins, C Nickerson-Nutter
Inflammation, Wyeth Research, Cambridge, MA, USA
Arthritis Research & Therapy 2007, **9(Suppl 3):**P1
(doi: 10.1186/ar2227)

Background IL-21 is secreted by activated T cells and modulates immune cell functions with both proinflammatory and anti-inflammatory effects. IL-21 receptor (IL-21R), homologous to IL-2Rβ and IL-4Rα, associates with the gamma common chain upon ligand binding. It was recently described that IL-21R is overexpressed in the inflamed synovial membrane and on leucocytes of rheumatoid arthritis patients.
Objective Previously we have shown that blockade of the IL-21 pathway with soluble IL-21R-Fc resulted in a reduction of clinical signs of arthritis in rodent models. To understand potential mechanisms of IL-21 regulation in arthritis, we analyzed serum immunoglobulin levels, and cytokine expression in the paws, serum, and collagen-restimulated splenocytes, in response to IL-21 pathway blockade.
Methods Arthritis was induced in DBA/1 male mice with bovine type II collagen. Animals were treated with either soluble mIL-21R-Fc, which neutralizes murine IL-21 bioactivity, with TNFRII-Fc or with control IgG. Spleens from each group of treated mice were cultured *in vitro* with collagen and assayed for cytokine secretion. Cytokines and anticollagen-specific IgG levels were also measured in the serum by ELISA. Cytokine mRNA levels in the paws were evaluated by quantitative PCR analysis.
Results Treatment of mice with IL-21R-Fc or TNFRII-Fc reduced clinical and histological signs of collagen-induced arthritis. IL-6 mRNA in paws and serum IL-6 levels were decreased after IL-21R-Fc treatment. IFNγ mRNA was increased in paws of IL-21R-Fc-treated mice. Collagen-specific spleen cell responses from IL-21R-Fc-treated mice exhibited increased IFNγ and IL-2, and reduced IL-6 and IL-17 levels. Serum levels of total IgG_1 were also reduced in response to IL-21R-Fc treatment.
Conclusion These data demonstrate a role for IL-21 in the modulation of collagen-specific T-cell responses and the pathology of arthritis, supporting a rationale for blockade of the IL-21 pathway in rheumatoid arthritis.

P2

Combined anti-inflammatory tritherapy using a novel small interfering RNA lipoplex successfully prevents and cures mice of arthritis

M Khoury[1,2], V Escriou[3,4,5,6], A Galy[7,8], R Yao[7,8], C Largeau[3,4,5,6], D Scherman[3,4,5,6], C Jorgensen[1,2,9], F Apparailly[1,2]
[1]Inserm, U 844, INM, Hôpital Saint Eloi, Montpellier, France; [2]Université Montpellier1, UFR de Médecine, Montpellier, France; [3]Inserm, U 640, Paris, France; [4]CNRS, UMR8151, Paris, France; [5]Université Paris Descartes, Faculté de Pharmacie, Paris, France; [6]Ecole Nationale Supérieure de Chimie de Paris, Paris, France; [7]Inserm, U 790, Genethon, Evry, France; [8]Université Paris-Sud 11, Orsay, France; [9]CHU Lapeyronie, service Immuno-Rhumatologie, Montpellier, France
Arthritis Research & Therapy 2007, **9(Suppl 3):**P2
(doi: 10.1186/ar2228)

Background TNFα is a key cytokine in rheumatoid arthritis (RA) physiopathology. We recently demonstrated that a new cationic liposome formulation allowed intravenous delivery of a small interfering RNA (siRNA) targeting TNFα and efficiently restoring the immunological balance in an experiment model of RA. Since 30% of patients do not respond to anti-TNF biotherapies, however, there is a need to develop alternative therapeutic approaches.
Objective Strong association of other proinflammatory cytokines with the pathogenesis of RA prompted us to investigate which cytokine other than TNFα could be targeted for therapeutic benefit using RNA interference.
Methods Two siRNA sequences were designed for IL-1β, IL-6 and IL-18 proinflammatory cytokines, and their efficacy and specificity were validated *in vitro* on J774.1 mouse macrophage cells, measuring both mRNA and protein levels following a lipopolysaccharide challenge. For *in vivo* administration, siRNAs were formulated as lipoplexes with the RPR209120/DOPE liposome and a carrier DNA, and were injected intravenously in DBA/1 mice having collagen-induced arthritis. The clinical course of the disease was assessed by paw thickness over time, and radiological and histological scores were obtained at euthanasia. The cytokine profiles were measured by ELISA in sera and knee-conditioned media. The immunological balance was assessed using antitype II collagen assays. The distribution of siRNAs was evaluated by fluorometry in GFP transgenic mice over time after anti-GFP siRNA lipoplex injections.
Results The designed siRNA sequences silenced 70–75% of the lipopolysaccharide-induced IL-1β, IL-6 and IL-18 mRNA expression in macrophages compared with a control siRNA. Each siRNA affected the targeted cytokine specifically, without modifying other proinflammatory cytokine mRNAs. In the collagen-induced arthritis model, weekly injections of siRNA lipoplexes significantly reduced the incidence and severity of arthritis, abrogating joint swelling, and destruction of cartilage and bone, in both preventive and curative settings. The most striking therapeutic effect was observed when

combining the three siRNAs targeting IL-1β/IL-6/IL-18 at once. Such tritherapy was associated with downregulation of both inflammatory and autoimmune components of the disease, and overall parameters were improved compared with the TNFα siRNA lipoplex-based treatment. The siRNA formulation was widely distributed, delivering the siRNA to several organs with a strong efficacy in the liver and spleen.

Conclusion Tritherapy targeting IL-1β/IL-6/IL-18 seems highly effective to reduce all pathological features of RA including inflammation, joint destruction and Th1 response. These data show that cytokines other than TNFα can be targeted to improve symptoms of RA and reveal novel potential drug development targets. The systemic administration of anticytokine siRNA cocktails as a lipoplex could represent a novel and promising anti-inflammatory alternative therapy in RA.

P3

An integrative genomics strategy for the identification of collagen-induced arthritis susceptibility genes

Saleh M Ibrahim
Section of Immunogenetics, University of Rostock, Rostock, Germany
Arthritis Research & Therapy 2007, **9(Suppl 3):**P3
(doi: 10.1186/ar2229)

Murine collagen-induced arthritis (CIA) is a chronic inflammatory disease bearing all the hallmarks of rheumatoid arthritis. CIA has been widely used to study the etiology, pathogenesis and new therapeutic approaches of rheumatoid arthritis. Previous studies have identified multiple quantitative trait loci (QTL) controlling different aspects of disease pathogenesis. However, progress in identifying the new susceptibility genes outside the MHC locus has been slow. With the advent of new global methods for genetic analysis such as large-scale sequencing, gene expression profiling, combined with classical linkage analysis, congenic and physical and *in silico* fine mapping, progress is considerably accelerating. Here we present preliminary data using an integrative genomics strategy to identify new putative susceptibility genes contributing to the pathogenesis of CIA.

The strategy is based on integrating a genome scan to identify QTLs linked to clinical disease phenotypes and subtraits in a cross between the CIA-susceptible DBA1/J and resistant FVB/NJ mouse strains. Additionally, gene expression profiling in target tissues and immune cells in parental strains and their F2 progeny is performed. Numerous classical QTL and expression QTL were identified. Master QTLs/expression QTLs controlling basic disease traits and subtraits were selected for further analysis using *in silico* tools; for example, haplotype sharing analysis, interspecies synteny analysis, congenic mapping, and pathway construction.

So far we have confirmed C5 as a putative susceptibility gene for the Cia2 locus on chromosome 2. We also identified the mitochondrial ATP8 as a novel susceptibility gene. We are also pursuing three additional QTLs on chromosomes 1, 5, 18 controlling leukocyte–endothelial cell adhesion, anticollagen antibody production and arthritis severity. Functional analysis of candidate genes for those loci is underway.

P4

Tumour necrosis factor-dependent inflammatory cartilage destruction

A Korb[1], B Tuerk[1], F Echtermeyer[2], T Pap[2], JS Smolen[1], K Redlich[1]
[1]Department of Rheumatology, Medical University of Vienna, Austria; [2]Division of Molecular Medicine of Musculoskeletal System, University of Muenster, Germany
Arthritis Research & Therapy 2007, **9(Suppl 3):**P4
(doi: 10.1186/ar2230)

Introduction Rheumatoid arthritis is a chronic inflammatory disorder that primarily affects the joints and results in the destruction of cartilage and subchondral bone by the inflamed synovium. Investigating the time course of cartilage destruction is of importance to better understand whether structural damage is reversible and when therapeutic intervention may be most effective.

Methods To study the time course and mechanisms of inflammatory destruction of articular cartilage, we used the human TNFα transgenic (hTNFtg) mouse model of rheumatoid arthritis. hTNFtg mice were sacrificed from weeks 2 to 14 in 2-week intervals, and sections of the hind paws were analyzed for histopathological changes. Specifically, the cartilage area and thickness were determined by histomorphometry, and proteoglycan loss was assessed by toluidin-blue staining. Inflammation was quantified by measuring the area of hyperplastic synovial tissue. The length of adhesion zones between pannus and cartilage were taken as measures for synovial attachment. To study the effects of proteoglycan loss on the attachment of synoviocytes *in vitro*, synovial fibroblasts were seeded onto isolated and IL-1-treated (2 ng/ml and 10 ng/ml, 24 hours) murine femoral heads, and the attachment was quantified by light microscopy.

Results In hTNFtg mice, synovial inflammation increased over time (0.025 mm^2 at week 2 versus 0.65 mm^2 at week 14; $P < 0.05$), with the most prominent increase between weeks 4 and 6 (0.029 mm^2 and 0.78 mm^2, respectively; $P < 0.05$). Loss of cartilage was seen only between weeks 2 and 4. After week 4, the cartilage area and thickness remained stable, arguing for a dissociation between cartilage degradation and pannus formation. As seen in toluidin-blue staining, a loss of proteoglycans occurred over time, which started at week 4 and peaked at week 8. There was a prominent attachment of pannus tissue to articular cartilage. Attachment started after week 4, peaked at week 10 and showed a decrease thereafter. This observation was supported by *in vitro* attachment data showing that early moderate loss of proteoglycans (as induced by 1 ng/ml IL-1) strongly enhanced attachment of synovial fibroblasts while an almost complete loss of proteoglycans (as induced by 10 ng/ml IL-1) did not facilitate fibroblast attachment.

P5

Histidine deficiency does not protect against aggrecan-induced arthritis

Gyorgy Nagy[1,2], Marianna Csilla Holub[1], Karoly Németh[1], Maria Pasztoi[1], Mercedesz Mazan[1], Agnes Koncz[3], Andras Falus[1,2], Edit Buzas[1]
[1]Department of Genetics, Cell and Immunbiology, Semmelweis University, Medical School, Budapest, Hungary; [2]Department of Rheumatology, Semmelweis University, Medical School, Budapest, Hungary; [3]Heim Pal Children's Hospital, Budapest, Hungary
Arthritis Research & Therapy 2007, **9(Suppl 3):**P5
(doi: 10.1186/ar2231)

Increased numbers of mast cells are found in the synovial tissues and fluids of patients with rheumatoid arthritis, and at sites of

cartilage erosion. Mast cell activation has been reported for a significant proportion of rheumatoid specimens. Because the mast cell contains potent mediators, including histamine, its potential contributions to the processes of inflammation and matrix degradation have recently become evident; thereafter, we investigated the potential protective effect of histamine deficiency against aggrecan-induced arthritis. To study the role of histamine in rheumatoid arthritis we investigated cartilage proteoglycan (aggrecan)-induced arthritis in histidine decarboxylase knockout (HDC-KO) mice, with complete lack of endogenously produced histamine. Aggrecan-induced arthritis was similar in HDC-KO and wild-type (WT) mice. Arthritis was even more severe in HDC-KO mice than in the WT animals 10 weeks following the immunization with aggrecan (arthritis score 1.2 ± 1.5 and 0 ± 0, respectively; $P = 0.01$). At later time points, arthritis scores were similar in both HDC-KO and WT mice. Since T-lymphocyte dysfunction has an important role in the pathogenesis of rheumatoid arthritis, next we investigated T-cell signal transduction and cytokine production in HDC-KO and WT mice. In the absence of histamine, elevated INFγ mRNA and protein levels of splenocytes ($P < 0.001$ and $P = 0.001$, respectively) were associated with a markedly increased (2.5-fold, $P = 0.0009$) nitric oxide (NO) production, compared with WT animals. Furthermore, histamine treatment decreased the NO production of splenocytes from both WT and HDC-KO mice ($P = 0.001$ and $P = 0.0004$, respectively). NO precursor (*Z*)-1-[2-(2-aminoethyl)-*N*-(2-ammonioethyl) amino]diazen-1-ium-1,2-diolate-diethylenetriamine (NOC-18) elicited IFNγ production ($P = 0.0002$), suggesting the role of NO in regulating IFNγ synthesis. The cytoplasmic Ca^{2+} concentration of unstimulated T cells and the T-cell activation-induced Ca^{2+} signal were increased in T cells from the HDC-KO mice ($P = 0.02$ and $P = 0.04$, respectively), while the T-cell activation-induced CD3 internalization was similar in both HDC-KO and WT animals. Our present data indicate that histamine deficiency does not protect against aggrecan-induced arthritis. The Th1 cytokine pattern and increased NO production may both contribute to the sensitivity of HDC-KO mice to aggrecan-induced arthritis. Furthermore, our data indicate that histamine, in addition to its direct effects on T-lymphocyte function, regulates cytokine production and T-cell signal transduction through regulating NO production.

Acknowledgements This work was supported by grants OTKA F 61030 and OTKA T 046468.

P6

Mast cells jump start K/BxN serum transfer arthritis via IL-1

PA Nigrovic[1], A Johnsen[1,2], BA Binstadt[2], PA Monach[1,2], M Gurish[1], Y Iwakura[3], D Mathis[2], C Benoist[2], DM Lee[1]
[1]Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, MA, USA; [2]Joslin Diabetes Center, Harvard Medical School, Boston, MA, USA; [3]Institute of Medical Science, University of Tokyo, Tokyo, Japan
Arthritis Research & Therapy 2007, **9(Suppl 3):**P6
(doi: 10.1186/ar2232)

Mast cell-deficient W/Wv mice are resistant to K/BxN serum transfer arthritis, and this resistance may be overcome by engraftment with mast cells. However, the pathways by which mast cells participate in arthritis remain unknown. Using a candidate mediator approach, we explored IL-1 as a potentially key mediator by which mast cells promote arthritis. As expected, IL-1α/β-deficient mice were completely resistant to arthritis. Short-term administration of exogenous IL-1 restored an attenuated arthritis course in these animals, consistent with an ongoing requirement for IL-1. Surprisingly, deficient W/Wv mice treated with IL-1 at disease induction displayed a full normal course of arthritis, demonstrating that exogenous IL-1 can bypass the need for mast cells. TNF proved unable to exert a similar effect. We therefore engrafted IL-$1^{-/-}$ bone marrow-derived mast cells (BMMC) into W/Wv animals and found that these animals displayed resistance to arthritis equivalent to nonengrafted W/Wv mice, consistent with an obligate role for IL-1 of mast cell origin. Exploring further the mechanisms by which mast cells may become activated in this IgG_1-driven model, we found that BMMC stimulated *in vitro* via FcγRIII elaborated IL-1, while BMMC lacking this receptor were unable to mediate arthritis upon engraftment into W/Wv recipients. While BMMC engrafted into W/Wv animals disproportionately populate the spleen, we excluded a contribution from this aphysiologic mast cell population via splenectomy and by documenting that systemic levels of IL-1 were not detectable during arthritis initiation in engrafted animals. We conclude that mast cells local to the joint and activated via FcγRIII promote K/BxN serum transfer arthritis by production of IL-1, an activity that appears delimited to the initiation of disease (the 'jump start').

Acknowledgements This work was supported by grant K08 AR051321 (PAN), Cogan Family Foundation and NIH R01 AI 59746-01 (DML).

Autoimmunity

P7

Disrupting Mer receptor tyrosine kinase expression prevents autoimmune chronic graft-versus-host disease

Wen-Hai Shao[1], Robert A Eisenberg[1], Philip L Cohen[1,2]
[1]Department of Medicine, Division of Rheumatology, University of Pennsylvania, Philadelphia, PA, USA; [2]Philadelphia VA Medical Center, Philadelphia, PA, USA
Arthritis Research & Therapy 2007, **9(Suppl 3):**P7
(doi: 10.1186/ar2233)

The Mer receptor tyrosine kinase mediates apoptotic cell phagocytosis and modulates macrophage cytokine production. Mer knockout mice (Mer-KO) have defective clearance of apoptotic debris and develop systemic lupus erythematosus-like autoimmune disease. Because of their spontaneous autoimmune propensity, we wondered whether Mer-KO mice (backcrossed 10 generations onto C57BL/6) might be particularly susceptible to the systemic lupus erythematosus-like autoimmunity induced by chronic graft-versus-host disease (GVHD). Using the well-established cGVH model (bm12→B6 mice), we were surprised to observe that the Mer-KO mice were protected from the development of GVHD. Mer-KO recipients of bm12 spleen cells failed to develop detectable anti-dsDNA and antichromatin autoantibodies, while the B6 hosts produced significant amounts of these antibodies that peaked at week 2. Slightly increased levels of rheumatoid factor were found in Mer-KO mice, although much lower than B6 control hosts. Mer-KO mice did not develop splenomegaly. The lack of autoantibody formation was not due to an absence of alloreactivity, because spleen cells from bm12 mice proliferated equally in response to irradiated wild-type and Mer-KO irradiated spleen cells. GVHD developed normally in F1 (Mer-KO x B6) hosts given bm12 donor cells, thus indicating that the failure to develop GVHD was not due to mer-KO-derived minor MHC genes. These findings indicate a hitherto unrecognized requirement for Mer in the genesis of alloreactivity-induced autoimmunity. Future experiments will determine whether this reflects the role of this kinase in recognition and phagocytosis of apoptotic cells, its function in regulating cytokine production, or perhaps a new function in the immune response.

P8

IL-21 and BAFF/BLyS synergize in stimulating plasma cell differentiation from human marginal zone B cells as well as from circulating peripheral blood B cells from autoimmune patients

Rachel Ettinger, Stefan Kuchen, Gary P Sims, Rachel Robbins, David Withers, Randy T Fischer, Peter E Lipsky
Autoimmunity Branch, NIAMS, National Institutes of Health, Bethesda, MD, USA
Arthritis Research & Therapy 2007, **9(Suppl 3):**P8
(doi: 10.1186/ar2234)

IL-21 promotes plasma cell (PC) differentiation while BAFF promotes B-cell survival. Here, we report that IL-21 synergizes with BAFF to elicit BLIMP-1 induction, PC differentiation and IgG production from a novel population of human splenic memory B cells. These human marginal zone analogue B cells are exquisitely sensitive to IL-21 and BAFF in the absence of further co-stimulation. The ability of IgG^+ marginal zone analogue to respond specifically and exclusively to IL-21 and BAFF demonstrates that they are uniquely poised to respond to antigen-independent signals and differentiate into IgG-producing PC, thereby replenishing serologic memory. Importantly, peripheral blood B cells from a portion of patients with systemic lupus erythematosus and rheumatoid arthritis were highly responsive to stimulation with IL-21 and BAFF. These data suggest that IL-21 and BAFF may be capable of inducing PC differentiation from memory B cells with autoreactive specificities and thereby contribute to autoimmunity.

P9

IL-33: a novel cytokine with proinflammatory properties

Gaby Palmer[1], David Moulin[1], Olivier Donzé[2], Dominique Talabot-Ayer[1], Cem Gabay[1]
[1]Service de Rhumatologie, HUG, Genève, Switzerland; [2]Apotech, Lausanne, Switzerland
Arthritis Research & Therapy 2007, **9(Suppl 3):**P9
(doi: 10.1186/ar2235)

Introduction IL-33 (or IL-1F11) was recently identified as a ligand for the previously orphaned IL-1 family receptor T1/ST2. IL-33 belongs to the IL-1 family of cytokines and upon binding to T1/ST2 induces intracellular signals similar to those of IL-1. Previous studies have established that IL-33 and T1/ST2 exert key functions in Th2 responses. Mast cells are known to express T1/ST2. Besides their well-known function in allergy and host defense, mast cells have recently been shown to play an important role in experimental models of arthritis.
Objective To examine the effect of IL-33 on P815 murine mastocytoma cells and primary mouse bone marrow-derived mast cells (BMMC).
Results IL-33 dose-dependently and time-dependently stimulated IL-6 secretion by P815 mastocytoma cells and BMMC. Both cell types express T1/ST2 as assessed by RT-PCR and FACS analysis. This effect was dependent on T1/ST2 binding. The addition of ST2-Fc on BMMC inhibited the stimulatory effect of IL-33, whereas etanercept had no effect. IL-1RAcP-Fc alone or in combination with ST2-Fc inhibited the effect of IL-33, indicating that IL-33 binds to these soluble receptors. In addition, IL-33 also induced IL-1β, TNFα, MCP-1, and PGD2 production in BMMC. By the RNase protection assay, we demonstrated that IL-33 increased IL-6 and IL-1β? mRNA expression. These effects of IL-33 appeared to occur independently of mast cell degranulation.
Conclusion The results of this study show for the first time that IL-33, a novel member of the IL-1 family of cytokines, stimulates the production of proinflammatory mediators by mast cells in addition to its effect on Th2 responses. These findings open new perspectives for the treatment of inflammatory diseases by targeting IL-33.

P10

Tolerogenic dendritic cells differentially modulate naïve and memory $CD4^+$ T cells

Amy E Anderson, Bethan Sayers, Julie Diboll, John D Isaacs, Catharien MU Hilkens
Clinical Immunotherapy Group, Department of Rheumatology, Newcastle University, UK
Arthritis Research & Therapy 2007, **9(Suppl 3):**P10
(doi: 10.1186/ar2236)

Background Dendritic cells (DC) can be alternatively activated to induce tolerogenic DC, making them a promising therapy for autoimmunity. Because naïve and memory T cells have different requirements for tolerisation, we tested the modulatory activity of tolerogenic DC on both subsets of T cells.
Methods Human monocyte-derived DC were matured with lipopolysaccharide (LPS-DC) or treated with dexamethasone, vitamin D3 and lipopolysaccharide (LPS-DexD3 DC) to obtain tolerogenic DC. DC were cocultured with allogeneic naïve or memory $CD4^+$ T cells. Primed T cells were rested and restimulated with LPS-DC or CD3/CD28 beads.
Results and conclusions LPS-DexD3 DC have reduced stimulatory capacity for both naïve and memory T cells. However, restimulation of T cells revealed a distinct difference between naïve and memory T cells that had been primed by LPS-DexD3 DC. Naïve T cells did not become anergic but were skewed to a regulatory phenotype (low IFNγ and high IL-10 production), whereas memory T cells were rendered hyporesponsive, with low proliferation and cytokine production. Thus, naïve and memory T cells are differently regulated by LPS-DexD3 DC. These data have implications for the use of tolerogenic DC vaccines as immunomodulators.

P11

Generation of dexamethasone and vitamin D3-treated human monocyte-derived dendritic cells with tolerogenic properties

Bethan Sayers, Muzlifah Haniffa, Julie Diboll, John Isaacs, Catharien Hilkens
Clinical Immunotherapy Group, Department of Rheumatology, Newcastle University, UK
Arthritis Research & Therapy 2007, **9(Suppl 3):**P11
(doi: 10.1186/ar2237)

Human monocyte-derived dendritic cells (DC) treated with dexamethasone and vitamin D3 (DexVitD3 DC) have tolerogenic properties and phenotype. DexVitD3 DC induce limited T-cell proliferation in an allogeneic mixed lymphocyte reaction and inhibit the T-cell proliferation induced by lipopolysaccharide and/or cytokine (TNFα/IL-1β) matured DC. Furthermore, T cells primed with DexVitD3 DC proliferate in response to restimulation with a distinct cytokine profile including significantly reduced production of IFNγ. DexVitD3 DC have characteristically low expression of the costimulatory molecules CD80/83/86 with high HLA-DR. Upon stimulation with lipopolysaccharide, DexVitD3 DC maintain their surface phenotype and produce large quantities of IL-10. We are currently characterising the cytokine profiles of DexVitD3 DC and the T cells they prime, as well as investigating whether these T cells have regulatory properties.

Cellular therapies are being explored as treatment for autoimmune diseases including rheumatoid arthritis. DexVitD3 DC have potential for future use in this field as well as being a useful tool to elucidate mechanisms of immune regulation.

P12

Arthritis induced by systemic autoimmunity against glucose-6-phosphate isomerase in normal mice

T Kamradt[1], L Bruns[1], O Frey[1], L Morawietz[2], D Schubert[3], A Taubner[1]

[1]Department of Immunology, Medical School, Friedrich-Schiller-Universität, Jena, Germany; [2]Department of Pathology, University Hospital Charité, Berlin, Germany; [3]German Center for Arthritis Research, Berlin, Germany

Arthritis Research & Therapy 2007, **9(Suppl 3):**P12
(doi: 10.1186/ar2238)

The antigens that trigger the pathogenic immune response in rheumatoid arthritis remain unknown. Until recently it was assumed that joint-specific antigens were the targets of arthritogenic T lymphocytes and B lymphocytes in rheumatoid arthritis. Consequently, murine models of arthritis are induced by immunization with either joint-specific antigens such as type II collagen or microbial products such as streptococcal cell wall. In the K/BxN T-cell receptor transgenic mouse model, arthritis is caused by a systemic autoimmune response to the ubiquitously expressed glycolytic enzyme glucose-6-phosphate isomerase (G6PI). More recently it was shown that G6PI immunization induces severe symmetrical peripheral polyarthritis in genetically unaltered DBA/I or SJL mice [1,2]. T cells are indispensable for both the induction and the effector phase of G6PI-induced arthritis. Arthritis is cured by depletion of CD4 cells. In contrast, antibodies and FcγR effector cells are necessary but not sufficient for G6PI-induced arthritis in genetically unaltered mice [1]. Both the induction and effector phase of arthritis induced by a systemic autoimmune response can be dissected and preventive and therapeutic strategies evaluated in this model.

References

1. Schubert D, Maier B, Morawietz L, Krenn V, Kamradt T: **Immunization with glucose-6-phosphate isomerase induces T-cell dependent peripheral polyarthritis in genetically unaltered mice.** *J Immunol* 2004, **172:**4503-4509.
2. Bockermann R, Schubert D, Kamradt T, Holmdahl R: **Induction of a B cell dependent chronic arthritis with glucose 6 phosphate isomerase.** *Arthritis Res Therapy* 2005, **7:**R1316-R1324.

P13

Regulation of eicosanoid production in peripheral blood mononuclear cells from patients with systemic sclerosis

Otylia Kowal-Bielecka[1], Krzysztof Kowal[2], Justyna Chwiecko[1], Stanislaw Sierakowski[1], Oliver Distler[3], Sylwia Chwiesko[1], Izabela Domyslawska[1], Steffen Gay[3]

[1]Department of Rheumatology and Internal Medicine, Medical University of Bialystok, Poland; [2]Department of Allergology and Internal Medicine, Medical University of Bialystok, Poland; [3]Center of Experimental Rheumatology, University Hospital, Zurich, Switzerland

Arthritis Research & Therapy 2007, **9(Suppl 3):**P13
(doi: 10.1186/ar2239)

Background Eicosanoids are arachidonic acid-derived mediators that play a key role in the regulation of inflammatory response. 5-Lipoxygenase (5-LOX)-derived leukotrienes are considered pro-inflammatory while 15-lipoxygenase (15-LOX)-derived products such as 15-hydroxyeicosatetraenoic acid (15-HETE) and lipoxins inhibit proinflammatory mediators including leukotrienes, and actively participate in the resolution of inflammation. It has been demonstrated that a temporal switch of arachidonic acid metabolism from predominant 5-LOX-derived to 15-LOX-derived products is crucial for the resolution of inflammation. We have recently shown that there is an imbalance between proinflammatory leukotrienes and anti-inflammatory lipoxins in the lungs of patients with systemic sclerosis (SSc)-related interstitial lung disease, which may favour chronic inflammation and fibrosis [1,2].

Objective To further investigate the role of eicosanoids in the pathogenesis of SSc through evaluation of: (1) the basal profile of eicosanoid synthesis, and (2) the pattern of eicosanoid biosynthesis in response to proinflammatory stimuli by peripheral blood mononuclear cells (PBMC) from patients with SSc in comparison with healthy people.

Methods Mononuclear cells were isolated from peripheral blood using density gradient centrifugation on Lymphoprep and cultured in fetal calf serum-supplemented RPMI medium at 37°C under 5% CO_2. Ionophore-stimulated production of 5-LOX-derived leukotriene E_4 (LTE4) and 15-LOX-derived 15-HETE was evaluated by means of enzyme immunoassay at predefined time points in basal conditions (without stimuli) as well as in response to TNFα. Ten patients with SSc (five diffuse and five limited cutaneous SSc) as well as five healthy controls were studied.

Results There were no significant differences in the basal production of LTE4 or 15-HETE between SSc patients and healthy controls. TNFα induced sequential changes in the production of eicosanoids, with an early (within 1 hour) increase in LTE4 followed by a delayed increase in 15-HETE in both SSc patients and healthy controls. PBMC from SSc patients responded to TNFα with significantly higher production of LTE4 in comparison with healthy controls ($P < 0.05$ at 1 hour), while there were no significant differences in TNFα-induced production of 15-HETE between SSc patients and controls.

Conclusions These preliminary results of our study indicate that the TNFα-induced eicosanoid synthesis is altered in PBMC from patients with SSc. Increased production of proinflammatory leukotrienes may contribute to the pathogenesis of SSc.

References

1. Kowal-Bielecka O, Distler O, Kowal K, *et al.*: **Elevated levels of leukotriene B4 and leukotriene E4 in bronchoalveolar lavage fluid from patients with scleroderma lung disease.** *Arthritis Rheum* 2003, **48:**1639-1646.
2. Kowal-Bielecka O, Kowal K, Distler O, *et al.*: **Cyclooxygenase- and lipoxygenase-derived eicosanoids in bronchoalveolar lavage fluid from patients with scleroderma lung disease: an imbalance between proinflammatory and antiinflammatory lipid mediators.** *Arthritis Rheum* 2005, **52:**3783-3791.

P14

Characterization of immunoglobulin mutations in humans with activation-induced cytidine deaminase deficiency

Nancy S Longo[1], Colleen Satorius[1], Anne Durandy[2], Peter E Lipsky[1]

[1]Autoimmunity Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD, USA; [2]Hopital Necker-Enfants Malades, Paris, France

Arthritis Research & Therapy 2007, **9(Suppl 3):**P14
(doi: 10.1186/ar2240)

Somatic hypermutation (SHM) is initiated in germinal center (GC) B cells expressing high levels of activation-induced cytidine deaminase (AID), which targets WRC (W = A/T, R = A/G) motifs generating a uracil:guanidine mismatch. During SHM, 73% of the mutations are attributed to AID and error-prone polymerase eta

(POLη). To characterize the nature of other mutations, 316 genomic nonproductive V(D)J rearrangements amplified and sequenced from three AID$^{-/-}$ patients were analyzed. The mutation frequency was 20-fold less than normals (0.09% versus 2%) but ~5 x 10^4-fold more than non-B cells. Reduced RGY (6% versus 27%) and WRC (6% versus 15%) motif mutations and decreased replacements in complementarity determining regions were attributed to the lack of AID. Reduced WA motif mutations (12% versus 23%) suggested POLη activity was decreased. Prominent G>C and A>T biases suggested that mutation and repair mechanisms occurred preferentially on one DNA strand. A high percentage of the mutations were G substitutions on the sense strand (43% versus 29% in normals), reflecting C mutational targeting on the opposite strand. These were primarily G to A transitions, suggesting that UNG activity was reduced even though UNG is upregulated in normal GC B cells and large GCs occur in AID$^{-/-}$ patients. The mutation pattern suggests that AID-independent C substitutions contribute a small proportion of SHM but lack the targeting provided by AID. Finally, a low level of SHM clearly can develop in the absence of AID; however, this mechanism is inefficient at altering the binding capacity of immunoglobulin heavy chain genes.

P15

CXCL13, CCL21 and CXCL12 are upregulated in mucosal-associated lymphoid tissue lymphomas in patients with Sjorgen's syndrome and cooperate in the maintenance of the immune response and malignant cell survival

F Barone[1], M Bombardieri[1], J Spencer[2], P Isaacson[3], F Humby[1], P Morgan[4], S Challacombe[4], G Valesini[5], C Pitzalis[1]
[1]Department of Rheumatology, GKT School of Medicine, London, UK; [2]Department of Immunology, GKT School of Medicine, London, UK; [3]Department of Immunology, UCL, London, UK; [4]Department of Oral Pathology and Medicine, GKT School of Medicine, London, UK; [5]Department of Reumatologia, La Sapienza, Roma, Italy
Arthritis Research & Therapy 2007, **9(Suppl 3):**P15
(doi: 10.1186/ar2241)

Background We previously demonstrated in minor salivary glands of patients with Sjogren's syndrome (SS-mSGs) that a true phenomenon of ectopic lymphoneogenesis takes places, with the formation of germinal centres (GCs), in association with the ectopic expression of the lymphoid chemokines (CKs) CXCL13 and CCL21. In Sjogren's syndrome major salivary glands (SS-MSGs) these structures are known to support clonal immunoglobulin heavy chain gene rearrangements and somatic hypermutation, favouring expansion and selection of autoreactive B-cell clones, clonally related to lymphomatous B-cell clones (responsible for lymphoma development in Sjogren's syndrome (SS)). The involvement of lymphoid CKs and CXCL12 in the development of lymphoid malignancies has been demonstrated. At present, however, no data on the expression of these CKs nor their cellular source within SS-MSGs and mucosal-associated lymphoid tissue lymphomas (MALT-Ls) from patients with SS have been described.
Objective To assess the specific contribution of lymphoid CKs in the organization of lymphoid proliferation within SS-MSGs and SS-MALT-Ls.
Methods We studied 12 SS-mSGs, four SS-MSGs with lymphoepithelial lesion and 20 SS-MSGs with non-Hodgkin B-cell MALT-Ls. In order to define the histological organization of the lymphoid infiltrate (reactive versus malignant areas) and identify the B-cell subpopulations infiltrating the glands, immunohistochemistry was carried out for cellular (CD21, bcl-2, bcl-6, IgD, CD20, CD3, CD68) and vascular markers (CD31, PNAd). On sequential sections, digital images for CXCL13, CCL21 and CXCL12 were analysed by thresholding positive staining in detected areas of interest. Digital image analysis estimated CK's volume fraction areas as a ratio between CXCL13, CCL21 and CXCL12 positive areas over malignant or reactive areas. CK's producing cells were identified by double staining on sequential sections. Mononuclear cells were isolated from two SS-MALT-L parotids following enzymatic digestion and stained for CD19, CD38, CD27, CD24, IgM, IgD, CD10, CD5, CD3, CD4, CD69, CD45RO, CXCR5, CXCR4 and CCR7. Finally, mRNA from total SS-MSGs and MALT-Ls and isolated B cells and T cells from MALT-Ls were analysed by RT-PCR for the transcript levels of CXCL13, CCL21 and CXCL12.
Results Reactive areas, characterized by T-cell/B-cell segregation, CD20$^+$IgD$^+$bcl-2$^+$ follicular B cells, presence of follicular dendritic cell networks in GCs and high endothelial venule formation were detected in 100% of MALT-Ls. A B-cell population (CD20$^+$IgD$^-$bcl-2$^+$) characterized by nuclear abnormalities and monocytoid appearance was consistently observed within the proliferating ducts and identified as the B-cell malignant component. FACS analysis on isolated MALT-L mononuclear cells showed B cells in diverse maturative stages (transitional, mature and memory B cells) and a B-cell population CD19^{pos}IgD^{low}CD24^{neg}CD27$^{low/neg}$CD5^{neg}CD10^{neg} not detectable in the controls. Ectopic expression of CXCL13 and CCL21 was observed in 100% of SS-MSGs and MALT-Ls. A significant increase in CXCL13's volume fraction analysis was observed both in SS-MSGs and SS-MALT-L reactive areas, as compared with SS-mSG follicular areas ($P < 0.05$ and $P < 0.05$, respectively). A strong difference in CXCL13 within the MALT-L reactive area compared with malignant areas was detected ($P < 0.001$). CCL21 was significantly increased in MALT-Ls compared with both SS-mSGs and SS-MSGs and was mainly confined to the T-cell area. CXCL12 was strongly expressed by ductal epithelial cells, vessels and reactive areas in SS-mSGs, SS-MSGs and MALT-Ls. Interestingly a significant increase in CXCL12 expression on MALT-L malignant areas as compared with reactive areas in SS-mSGs and SS-MSGs was detected ($P < 0.01$ and $P < 0.05$). RT-PCR analysis showed increased CXCL13 and CCL21 level in SS-MSGs compared with MALT-Ls and SS-mSGs, while strong upregulation in CXCL12 transcript in MALT-Ls as compared with SS-mSGs and SS-MSGs was detected.
CXCL13, CCL21 and CXCL12 were detected on CD68$^+$ cells by immunohistochemistry, while CD20 and CD3, CXCL13 and CCL21double staining and mRNA analysis on MALT-extracted T and B lymphocytes showed negligible expression of the two CKs in MALT-L-extracted lymphocytes. Interestingly we identified strong CXCL12 expression on CD19$^+$ MALT-L isolated cells both at the protein and mRNA level and on ductal epithelial cells in close contact with the malignant B-cell infiltration. In agreement with this increase, we demonstrated a significant downregulation of CXCR4 on MALT-L-isolated B cells, likely to be the result of CXCR4 internalization upon CXCL12 ligation.
Conclusion In SS-MALT-Ls a strong upregulation of the lymphoid CKs CXCL13 and CCL21 takes place and is associated with the organization of the reactive areas involved in the maintenance of the autoimmune process within the malignancy. These findings support a contributory role for CXCL13 and CCL21 in the pathogenesis of MALT lymphomas in SS. These results imply that the CK/CK$^-$ receptor axis is functional in MALT-L malignant cells, and suggest that the glandular microenvironment, B-cell receptor signalling upon antigen engagement and autocrine signals from the same malignant population concur in MALT lymphomagenesis, triggering local activation of malignant B cells and favouring their survival and expansion.

P16

Fine-specificity of the antibodies against citrullinated protein response is influenced by shared epitope alleles

KN Verpoort[1], K Cheung[2], A Ioan-Facsinay[1], AHM van der Helm-van Mil[1], RRP de Vries[3], FC Breedveld[1], TWJ Huizinga[1], GJM Pruijn[2], REM Toes[1]

[1]Department of Rheumatology, Leiden University Medical Center, The Netherlands; [2]Department of Biomolecular Chemistry, Radboud University Nijmegen, The Netherlands; [3]Department of Immunohaematology & Blood Transfusion, Leiden University Medical Center, The Netherlands

Arthritis Research & Therapy 2007, **9(Suppl 3):**P16
(doi: 10.1186/ar2242)

Objective In classic studies on the genetic background of antibody production, MHC has been shown to act as the most prominent immune-response gene that controls the magnitude and the specificity of antibody production. The strongest genetic risk factor for rheumatoid arthritis (RA), the human MHC, HLA-DRB1 shared epitope (SE) alleles, predisposes for antibodies against citrullinated proteins (ACPA). ACPA levels are higher in SE-positive than in SE-negative RA patients. The present study determines whether SE not only influences the magnitude, but also the specificity of the ACPA response.
Methods In two independent cohorts of anti-CCP2-positive RA patients ($n = 206$ and $n = 214$ patients, respectively), serum antibodies against a citrullinated peptide derived from vimentin (cVim) and antibodies against a citrullinated fibrinogen peptide (cFibr) were determined by ELISA. HLA-DRB1 genotyping was performed.
Results In the first cohort, SE alleles were significantly associated with the presence of antibodies against cVim (OR = 4.55, 95% CI = 1.78–12.9) and not significantly with the presence of antibodies against cFibr (OR = 1.81, 95% CI = 0.78–4.13). These results were replicated in the second cohort (OR = 4.13, 95% CI = 1.68–10.3 and OR = 1.08, 95% CI = 0.34–3.23, respectively).
Conclusion In two cohorts of ACPA-positive RA patients, SE alleles predispose for the development of antibodies against cVim, and not for the development of antibodies against cFibr. These data indicate that SE alleles act as 'classic' immune-response genes in the ACPA response, as they influence both the magnitude and the specificity of this RA-specific antibody response.

Rheumatoid arthritis

P17

Activation of synovial fibroblasts by laminin-1 and transforming growth factor beta induces expression of stromelysins independently of TNFα, IL-1β or NF-κB

Katrin Warstat[1], Thomas Pap[2], Gerd Klein[3], Steffen Gay[4], Wilhelm K Aicher[1]

[1]Center for Medical Research (ZMF), Department of Orthopedic Surgery, Eberhard-Karls-University Medical School, Tübingen, Germany; [2]Division of Molecular Medicine of Musculoskeletal Tissue, Department of Orthopedics, Münster University Hospital, Münster, Germany; [3]Center for Medical Research (ZMF), Section for Transplantation Immunology, University of Tübingen, Tübingen, Germany; [4]WHO Center for Experimental Rheumatology, University Hospital Zürich, Zürich, Switzerland

Arthritis Research & Therapy 2007, **9(Suppl 3):**P17
(doi: 10.1186/ar2243)

Recently it was shown that attachment of synovial fibroblasts (SF) from rheumatoid arthritis patients to laminin-111 (LM-111) induced an elevated expression of stromelysin-1 (MMP-3). We therefore investigated the regulation of additional matrix metalloproteinases (MMPs) and their specific tissue inhibitors of matrix metalloproteinases (TIMPs) by attachment to LM-111 in the presence of transforming growth factor beta (TGFβ). Changes in steady-state mRNA levels encoding TIMPs and MMPs were investigated by quantitative RT-PCR. Production of MMPs and cytokines was monitored by a multiplexed immunoarray or by ELISA. Signal transduction pathways were studied by immunoblotting. Attachment of SF to LM-111 in the presence of TGFβ induced significant increases in stromelysin-1 mRNA (12.35-fold, $P < 0.001$) and protein (mean 62 ng/ml, sixfold, $P < 0.008$). Expression of stromelysin-2 (MMP-10) mRNA (11.68-fold, $P < 0.05$) and protein (54 ng/ml, 20-fold, $P \geq 0.02$) was significantly activated as well. All other TIMPs and MMPs investigated failed to show this LM-111-facilitated TGFβ response. Induction of stromelysin-1 and stromelysin-2 was associated with the activation of transcription factors c-fos and Egr-1, but phosphorylation of NF-κB was not observed. Further, LM-111-activated and TGFβ-activated SF failed to produce remarkable amounts of IL-1β or TNFα. We conclude that costimulation of synovial fibroblasts by LM-111 together with TGFβ suffices to induce significant expression of MMP-3 and MMP-10 by SF and that this induction is independent of phosphorylation of NF-κB.

P18

Tumor necrosis factor polymorphisms in psoriatic arthritis: association with the promoter polymorphism TNF-857 independent of the PSORS1 risk allele

K Reich[1], U Hüffmeier[2], IR König[3], J Lascorz[2], J Lohmann[4], J Wendler[5], H Traupe[6], R Mössner[1], A Reis[2], H Burkhardt[7]

[1]Georg-August-University Göttingen, Germany; [2]Institute of Human Genetics, University Erlangen–Nuremberg, Germany; [3]Institute of Medical Biometry and Statistics, University of Lübeck, Germany; [4]Psoriasis Rehabilitation Hospital, Bad Bentheim, Germany; [5]Rheumatologische Schwerpunktpraxis, Erlangen, Germany; [6]Department of Dermatology, University of Münster, Germany; [7]Division of Rheumatology, Johann Wolfgang Goethe University Frankfurt am Main, Germany

Arthritis Research & Therapy 2007, **9(Suppl 3):**P18
(doi: 10.1186/ar2244)

Background Single nucleotide polymorphisms (SNPs) of the *TNF* gene at positions −238 and −308 have earlier been associated with psoriasis vulgaris and psoriatic arthritis (PsA). However, a strong linkage disequilibrium at the chromosomal region 6p21 renders the interpretation of these findings difficult since also other risk factors for psoriasis (PSORS1) than SNPs of the *TNF* gene have bee mapped to that particular region. Therefore, in this study several SNPs of the *TNF* gene and of its neighbouring lymphotoxin α (*LTA*) gene were analysed independently and dependently on carrying the PSORS1 risk allele.
Methods SNPs in the promoter of the *TNF* gene (−238G/A, −308G/A, −857C/T, −1031T/C), and one SNP of the *LTA* gene (+252A/G), of the *TNLFRSF1A* gene (+36A/G) and of the *TNLFRSF1B* gene (+676T/G), respectively, were genotyped in 375 psoriasis patients, 375 PsA patients, and 376 controls. The tryptophan–tryptophan–cysteine–cysteine haplotype of the *CCHCR1* gene (*CCHCR1*WWCC*) was used to estimate the genetic impact of the PSORS1 risk allele.
Results Whereas an earlier-described association of allele *TNF*−238A* with psoriasis could be confirmed, our study revealed that this association was completely dependent on concomitant carriage of the PSORS1 risk allele. For PsA, but not psoriasis

vulgaris without joint manifestations, strong association with the allele *TNF*–857T* was detected (OR = 1.956; *P* value corrected for multiple testing, $P_{corr} = 0.0025$) also in patients negative for the PSORS1 risk allele.

Conclusions Our results indicate genetic differences between psoriasis vulgaris patients with and without joint manifestation. While the previously reported association between *TNF*–238A* and psoriasis seems to primarily reflect linkage disequilibrium with PSORS1, *TNF*–857T* may represent a risk factor for PsA independent of PSORS1. A potential pathophysiologic relevance of the elucidated genetic association is further suggested by previously reported experimental evidence for a functional impact of the respective TNF polymorphism on TNFα expression levels.

P19

Safety and diagnostic value of medical arthroscopy: retrospective analysis of 100 medical arthroscopies performed by a rheumatologist

Ved Chaturvedi
Rheumatology, Research & Referrral Army Hospital, New Delhi, India
Arthritis Research & Therapy 2007, **9(Suppl 3):**P19
(doi: 10.1186/ar2245)

Background There are few rheumatologists who now use the technique of arthroscopy; in most centers, referral is made to an orthopedic surgeon who specializes in this technique. It has become clear in recent years that the synovium is the primary site of inflammation and the major effecter organ in a variety of joint diseases including rheumatoid arthritis [1]. The present retrospective study was primarily carried out to assess the safety of this procedure in the hands of a rheumatologist and also to assess the utility of the procedure in various joint diseases.

Objective To evaluate the safety and diagnostic value of medical arthroscopy performed by a rheumatologist.

Methods Decisions for performing arthroscopy were taken when a detailed clinical history and relevant rheumatological investigations failed to arrive at a definite diagnosis. Initial assessment included detailed clinical history and relevant diagnostic tests for rheumatic diseases. Routine blood count, bleeding time, clotting time and biochemical parameters were also determined. After obtaining written consent, arthroscopies were performed as an outpatient department procedure using a 4 mm arthroscope with 0° and 30° viewing. All the procedures of arthroscopy were performed by a single rheumatologist. Procedures were done in a minor operating theatre under local anesthesia using strict aseptic precautions. Xylocaine 2% and bupivacaine 0.5% were used as local anesthetic agents for skin portal and intraarticular anesthesia, respectively. A tourniquet was not used during the procedures and the skin portal was not stitched. Synovial biopsies taken during procedures were subjected to only histopathological examination. All the procedures were recorded on a video-cassette.

Results See Tables 1 and 2. None of the patients developed any complications during the procedure or after 6 months of follow-up. None of the patients experienced any discomfort during the procedure except for a mild stretching sensation experienced by male patients. Females tolerated the procedure better than males.

Conclusion Arthroscopy using a 4 mm scope in the hands of rheumatologists is a safe daycare procedure. It can be performed without a tourniquet. The local anesthetic agent bupivacaine provides an excellent local anesthesia. Females tolerate the procedure better than males. In a few cases arthroscopy helped in arriving at a final diagnosis but many patients remained undiagnosed. Both the rheumatologists and the pathologists require further experience in this field.

Table 1 (abstract P19)

Patient characteristics	
Total number of patients	50
Number of males	39
Number of females	11
Mean age of patients (years)	35.5

Table 2 (abstract P19)

Postarthroscopy diagnosis	
Polyarticular gout	3
Monoarticular gout	2
Oligoarticular gout	3
Tubercular monoarthritis	3
Villonodular synovitis	1
Synovial chondromatosis	1
Histoplasmosis	1
Osteoarthritis	1
Septic arthritis	1
Rheumatoid arthritis	5
Nonspecific chronic synovitis	29

Reference

1. Tak PP: **Analysis of synovial biopsy and sample: opportunities and challenges.** *Ann Rheum Dis* 2000, **59:**929-930.

P20

Investigation of the role of the p38 MAPK α and δ isoforms in nonresponse to tumour necrosis factor blockade in the synovium of rheumatoid arthritis patients

LR Coulthard[1], LD Church[1], RJ Mathews[1], S Churchman[1], L Dickie[1], M Buch[1], R Reece[1], A English[1], AW Morgan[1], S Gay[2], P Emery[1], MF McDermott[1]
[1]Leeds Institute of Molecular Medicine, St James's University Hospital, Leeds, UK; [2]Department of Rheumatology, University Hospital, Zurich, Switzerland
Arthritis Research & Therapy 2007, **9(Suppl 3):**P20
(doi: 10.1186/ar2246)

Background Thickening of the synovial membrane with proliferation of macrophage-like and fibroblast-like synoviocytes is observed in affected joints of rheumatoid arthritis (RA) patients, as well as extensive synovial infiltration of inflammatory cells. TNF plays a key role in driving the pathogenesis and persistence of RA. Blockade of TNF, using current biologics, has had profound therapeutic effects; nevertheless, between 30% and 40% of RA patients do not respond to this treatment. The mechanisms of response and nonresponse to biologic therapy remain unclear. p38 MAPK is present in the rheumatoid synovium and thought to play a role in the pathogenesis of RA, suggested by evidence that p38 is required for TNF-induced inflammatory processes. There are four p38 MAPK isoforms, α, β, γ and δ, which are found in varying levels in inflammatory cells. Although most research has to date focused on p38α, it has been demonstrated in the synovium that the functional protein produced from the L1 retrotransposable element can specifically induce p38δ. This suggests a possible second p38-mediated pathway of joint destruction in RA that may not be

blocked by anti-TNF therapy. This study aimed to correlate the expression of p38δ in the rheumatoid synovium with clinical response and nonresponse to the anti-TNF biologic, infliximab (Remicade).

Methods We examined the expression of p38 MAPK isoforms (α and δ) pre and post infliximab therapy in the synovium of RA patients, to compare clinical response with nonresponse. All patients entered into this study have failed at least two disease-modifying drugs, and fulfil the revised 1987 ACR criteria for RA. The effect of TNF blockade in five responders and three non-responders on the p38 MAPK α and δ isotypes was studied; expression and activation (phospho-p38) in synovial biopsies of responders and nonresponders was measured by immuno-histochemistry using a semiquantitative scoring system, at baseline and approximately week 16 of therapy. A therapeutic effect was determined by patients achieving an ACR20 response at week 14, and the Wilcoxon signed rank test was performed to assess significance of changes in expression levels post treatment.

Results In responders, p38α, p38δ and phospho-p38 expression were all significantly decreased post infliximab treatment in the synovial sublining layer ($P < 0.05$). A similar trend was seen in the synovial lining layer of responders, with a decrease in expression of p38α and p38δ and phospho-p38 post infliximab treatment in four of five responders, although these results did not reach statistical significance. p38δ and phospho-p38 in the sublining layer and phospho-p38 in the lining layer increased, or remained high, in all three nonresponders tested, and this tendency was the opposite to that seen in the responders.

Conclusion In the RA synovium, expression and activation of p38α and p38δ in the sublining layer correlated with response, and increased p38δ expression and activation was associated with nonresponse. A flow cytometric-based investigation of p38 isoform expression/activation in peripheral blood mononuclear cells has been initiated.

P21

The synovial cadherin (cadherin-11) promotes intercellular motility

Hans P Kiener, Christopher S Stipp, Philip G Allen, David M Lee, Michael B Brenner

Department of Medicine, Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Arthritis Research & Therapy 2007, **9(Suppl 3):**P21
(doi: 10.1186/ar2247)

Cadherin-11 is a homophilic adhesion molecule that is expressed on fibroblast-like synoviocytes. Cadherins mediate tissue morphogenesis and architecture. Cadherin cell adhesion contacts are actively remodeled and impact cell movement and migration over other cells. To determine the molecular mechanisms that contribute to cadherin-11-dependent cell migration, we generated cadherin-11 mutants. We found that expression of a mutant cadherin-11 lacking the cytoplasmic juxtamembrane domain (JMD) diminished the turnover of α-catenin at adherens junctions as measured by fluorescence recovery after photobleaching. This resulted in markedly diminished cell intercalation into monolayers reflecting reduced cadherin-11-dependent cell motility on other cells. Furthermore, the actin cytoskeleton in cadherin-11 ΔJMD cells revealed a more extensive cortical F-actin ring that correlated with significantly higher levels of activated Rac1. Together, these studies implicate the cadherin-11 cytoplasmic JMD as a regulator of intercellular motility and cellular rearrangement in multicellular clusters and provide insight into a critical pathway that determines the behavior of fibroblast-like synoviocytes in arthritis, especially rheumatoid arthritis.

P22

Cadherin-11 regulates synovial fibroblast behavior in health and disease

David M Lee[1], Hans P Kiener[1], Sandeep K Agarwal[1], Erika H Noss[1], Gerald FM Watts[1], Osamu Chisaka[2], Masatoshi Takeichi[3], Michael B Brenner[1]

[1]Department of Medicine and Division of Rheumatology, Immunology and Allergy, Brigham & Women's Hospital, Harvard Medical School, Boston, MA, USA; [2]Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Yoshida-honmachi, Kyoto, Japan; [3]RIKEN Center for Developmental Biology, Minatojima-Minamimachi, Chuo-ku, Kobe, Japan

Arthritis Research & Therapy 2007, **9(Suppl 3):**P22
(doi: 10.1186/ar2248)

Cadherin adhesion molecules, structurally typified by their immunoglobulin-like extracellular (cadherin) domains rigidified as an extended chain by interdomain calcium binding, mediate cellular adhesion by binding a cadherin of the same type on an adjacent cell (homophilic adhesion). During embryonic development, cadherins provide a basis for cell sorting, aggregation and resultant tissue morphogenesis and they are thought to contribute to tissue maintenance postnatally. In tumors, cadherins have been implicated in tissue extension, migration and invasion. Knowing these processes are active in the synovium both at baseline and in the context of the hyperplastic and invasive synovial tissue in inflammatory arthritis, we hypothesized that a cadherin may play a role in regulating this tissue behavior. Using mouse models, we demonstrate that synovial tissue fibroblasts express cadherin-11. We find that cadherin-11 null mice display a hypoplastic synovial lining and display attenuated arthritic responses to K/BxN serum transfer arthritis. Moreover, we find a remarkable decrease in synovial pannus invasion into cartilage. These observations reveal a role for cadherin-11 in the molecular regulation of the organized behavior of the synovial fibroblast both in health and disease.

P23

Toll-like receptor 9 agonists and IL-15 promote activation, proliferation, secretion of proinflammatory cytokines and differentiation of B cells isolated from bone marrow of rheumatoid arthritis patients

W Rudnicka[1], E Warnawin[1], T Burakowski[1], M Bik[1], E Kontny[1], M Chorazy-Massalska[1], A Radzikowska[1], M Buler[1], P Maldyk[2], W Maslinski[1]

[1]Department of Pathophysiology and Immunology, and [2]Clinic of Orthopaedic Surgery, Institute of Rheumatology, Warsaw, Poland

Arthritis Research & Therapy 2007, **9(Suppl 3):**P23
(doi: 10.1186/ar2249)

Background Accumulating data indicate that bone marrow (BM) in rheumatoid arthritis (RA) patients participates in the pathogenesis of RA as a site of proinflammatory cytokine overproduction, lymphocyte homing and cell activation. IL-1b, IL-6, TNFα and IL-15 are among the proinflammatory cytokines elevated in RA in comparison with osteoarthritis (OA) patient BM. At present, it is unclear why the RA BM microenvironment produces more proinflammatory cytokines than OA BM. One possibility is that, in addition to physiological production, exogenous and/or endo-genous factors further enhance innate immune responses in RA

BM. Importantly, our recent data indicate that RA BM-derived B cells express functional Toll-like receptor 9 (TLR9), and therefore could respond to TLR9 agonist stimulation.

Objectives To test the effects of TLR9 agonists (oligodeoxynucleotides containing unmethylated CpG sequences (CpG-ODN)) on RA BM-derived B cells, and to estimate the presence of bacterial DNA in BM from RA and OA patients.

Methods BM samples, obtained from RA patients during joint replacement surgery, served for bone marrow mononuclear cell isolation. Mature ($CD20^+$) B cells were next purified from bone marrow mononuclear cells using a magnetic cell separation technique (MACS) and cultured *in vitro* in the presence of agonistic CpG-ODN or control GpC-ODN alone or with addition of IL-15. mRNA was isolated from purified B cells using Trizol and served as the template for RT-PCR analysis. The presence of bacterial DNA in BM plasma and bone marrow mononuclear cells was evaluated by DNA extraction and PCR amplification using primers specific for eubacterial 16S-ribosomal RNA. Flow cytometry was applied to assess the expression of intracellular proteins (TLR9 and proliferation marker Ki-67) and surface activation markers (CD86, CD54) on B lymphocytes. B-cell populations were distinguished according to CD19, CD20, CD27 and CD138 molecule expression. Specific ELISAs were used to measure TNFα and IL-6 in supernatants from cultured cells.

Results We found that BM-derived B lymphocytes isolated from RA patients express TLR9 at the mRNA and protein levels. Importantly, these TLR9 are functional. Stimulation of B cells by CpG-ODN, but not control GpC-ODN, in a dose-dependent manner enhanced expression of activation markers (CD86 and CD54), and stimulated IL-6 and TNFα secretion and cell proliferation *in vitro*. The specificity of CpG-ODN-triggered stimulation was confirmed in blocking experiments where cells cultured in the presence of chloroquine, a known inhibitor of TLR9 signaling, failed to respond to CpG-ODN. Moreover, in the presence of IL-15, stimulatory activities of TLR9 agonists were further enhanced. CpG-ODN, but not IL-15 alone, trigger differentiation of sorted B cells into $CD19^+CD20^+CD27^{high}$ cells. Simultaneous addition of CpG-ODN and IL-15 promoted further differentiation of $CD19^+CD20^+CD27^{high}$ cells toward plasma cells, as judged by upregulation of CD138 and downregulation of CD19 and CD20 expression. Finally, the presence of highly conserved regions of the eubacterial 16S-ribosomal RNA gene in samples of BM from RA patients was found at significantly higher frequency when compared with OA patients.

Conclusion Our data indicate that BM of RA patients may represent an important secondary lymphoid organ that, especially during ongoing infection with the presence of bacterial DNA in blood and ultimately in BM, actively participates in the pathogenesis of RA.

P24

Class switched memory B cells are enriched in the synovium after rituximab treatment

Burkhard Möller, Istvan Vajtai, Sabine Adler, Stefan Eggli, Hans-Rudolf Ziswiler, Clemens Dahinden, Peter M Villiger
Inselspital – University Hospital Bern, Department for Rheumatology and Clinical Immunology, Bern, Switzerland
Arthritis Research & Therapy 2007, **9(Suppl 3):**P24
(doi: 10.1186/ar2250)

A 49-year-old female was diagnosed with polyarticular, rheumatoid factor-positive juvenile idiopathic arthritis at the age of 15. Advanced destruction required bilateral knee joint replacement 8 years later. Persistent inflammatory activity led to rituximab treatment (1,000 mg on days 1 and 15) in an advanced disease stage. Peripheral blood analyses showed immediate B-cell depletion. Synovial tissue histology 8 months later in the early reconstitution phase revealed scattered follicular-like infiltrates including $CD20^+$ B cells. Flow cytometry of homogenized tissue showed $CD19^+$ B cells predominantly representing an IgD^-CD27^+ class switched memory phenotype, which was contrasted by >90% IgD^+CD27^- peripheral blood B cells. This observation, which indicates either local persistence or early B-cell repopulation and a composition of synovial B cells discordant to peripheral blood, could have an impact on the future development of monitoring strategies after rituximab treatment in arthritis.

P25

Evidence for the role of CD74 in innate immunity, arthritis, and the action of migration inhibitory factor

Eric Morand, Lanie Santos, Pamela Hall, Michael Hickey
Centre for Inflammatory Diseases, Monash University, Melbourne, Australia
Arthritis Research & Therapy 2007, **9(Suppl 3):**P25
(doi: 10.1186/ar2251)

Recent studies have identified CD74, the cell surface form of the class II-associated invariant chain, as a binding site for the cytokine macrophage migration inhibitory factor (MIF). MIF is implicated in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus, but a functional relationship between MIF and CD74 in inflammation has not been demonstrated. We used $CD74^{-/-}$ mice to examine the role of this molecule in cellular and *in vivo* inflammatory responses, using innate immune response-dependent models to avoid confounding by the role of MHC Ii in adaptive immunity.

MIF has a demonstrated role in macrophage responses to endotoxin. LPS induced IL-1, IL-6, TNF and MIF release from WT bone-marrow macrophages. Bone-marrow macrophages lacking CD74 released significantly lower amounts of IL-1 and IL-6, but no difference in TNF release was evident. LPS-induced MIF release by WT and $CD74^{-/-}$ bone-marrow macrophages was also similar. Murine K/BxN serum-transfer arthritis is MIF dependent [1], and also requires the induction of IL-1. Compared with WT mice, arthritis severity (clinical score) was significantly lower in mice lacking CD74 ($P < 0.05$). These data imply but do not demonstrate a role of CD74 in MIF-dependent inflammatory responses. To confirm the role of CD74 in responses to MIF *in vivo*, we examined a recently described action of MIF – the induction of leukocyte trafficking [2]. rhMIF was injected into mouse cremaster tissue and leukocyte–endothelial interactions examined using intravital microscopy. MIF induced leukocyte adhesion and emigration *in vivo* in WT mice. In $CD74^{-/-}$ mice, in contrast, this response could not be elucidated.

These data demonstrate for the first time the role of CD74 in innate immune responses and inflammatory arthritis, and demonstrate for the first time *in vivo* the requirement for CD74 for the action of MIF. A functional role for CD74 in MIF responses in inflammation is strongly supported.

References

1. Santos LL, Dacumos A, Mackay CR, Morand EF: **Macrophage migration inhibitory factor (MIF)-deficient mice are protected from K/BxN serum transfer arthritis [abstract].** *Arthritis Rheum* 2006, **54:**S352.
2. Gregory JL, Morand EF, McKeown SJ, *et al.*: **Macrophage migration inhibitory factor induces macrophage recruitment via CC chemokine ligand 2.** *J Immunol* 2006, **177:**8072-8079.

P26

Nitric oxide differentially regulates T-cell function in rheumatoid arthritis and systemic lupus erythematosus

Gyorgy Nagy[1,2], Joanna M Clark[3], Edit Buzas[2], Claire L Gorman[3], Pal Geher[1], Andras Perl[4], Andras Falus[2], Andrew P Cope[3]

[1]Department of Rheumatology and [2]Department of Genetics, Cell and Immunobiology, Semmelweis University, Medical School, Budapest, Hungary; [3]Kennedy Institute of Rheumatology Division, Faculty of Medicine, Imperial College, London, UK; [4]Department of Medicine, State University of New York, College of Medicine, Syracuse, NY, USA

Arthritis Research & Therapy 2007, **9(Suppl 3):**P26
(doi: 10.1186/ar2252)

Experimental and clinical evidence for T-cell involvement in the pathogenesis of rheumatoid arthritis (RA) is compelling, and points to a local dysregulation of T-cell function in the inflamed joint. Nitric oxide (NO) has been shown to regulate T-cell function under physiological conditions, but overproduction of NO may contribute to lymphocyte dysfunction in RA. NO has recently been recognized as a key signaling intermediate for T-cell activation and mitochondrial biogenesis. NO is synthesized from L-arginine by NO synthetases (NOS). Three distinct isoforms of NOS are known, including neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) enzymes. We previously detected the expression of eNOS and nNOS and the absence of iNOS in human PBL, while during inflammation macrophages and monocytes express iNOS. Systemic lupus erythematosus (SLE) is a systemic autoimmune disease of unknown origin characterized by the involvement of multiple organs. Several studies carried out on patients with both RA and SLE have documented increased endogenous NO synthesis, but its contribution to T-lymphocyte mitochondrial biogenesis and T-cell dysregulation is not known.

We investigated the role of NO in T-cell mitochondrial biogenesis in RA and SLE.

The mitochondrial mass, NO production and cytoplasmic Ca^{2+} levels were measured by flow cytometry. Mitochondria were visualized using transmission electron microscope.

T cells from RA patients produce >2.5 times more NO than T cells from healthy donors ($P < 0.001$). Unexpectedly, the mitochondrial mass was found to be similar in RA and control T cells ($P = 0.65$), whilst increased NO production was associated with increased cytoplasmic Ca^{2+} concentrations in RA T cells ($P < 0.001$). We observed that T-cell NO production decreased in most RA patients following anti-TNF treatment. Although lupus T cells produced comparable amounts of NO to normal T cells, lupus monocytes produced twice as much NO as normal monocytes ($P = 0.015$). We also observed increased mitochondrial mass ($47.7 \pm 2.8\%$; $P = 0.00017$) and increased cytoplasmic ($38 \pm 6.4\%$; $P = 0.0023$) Ca^{2+} content in T cells from SLE patients when compared with control donors. Electron microscopy revealed that T cells of lupus patients contained 8.76 ± 1 mitochondria, while control donors contained 3.18 ± 0.28 mitochondria per cell ($P = 0.0009$). In addition, lupus lymphocytes harbor several-fold enlarged megamitochondria. These data suggest that monocytes are the primary source of NO in SLE, while T lymphocytes are the primary source of NO in RA. Although the iNOS pathway is not as rapid as eNOS or nNOS, it is thought to be capable of generating much larger quantities of NO (nanomolar range) than the constitutive NOS isoforms (picomolar range), explaining the differences in T-cell mitochondrial biogenesis in SLE and RA. Since mitochondria can take up, store and release Ca^{2+}, increased mitochondrial mass may account for altered Ca^{2+} handling in SLE. Furthermore increased NO production may contribute to T-cell dysfunction in both SLE and RA.

Acknowledgement This work was supported by grant OTKA F 61030.

P27

Circulating levels of IL-7 in rheumatoid arthritis

S Churchman, S Field, C Burgoyne, A Brown, P Emery, F Ponchel

Leeds Institute of Molecular Medicine, Section of Rheumatology, University of Leeds, Leeds, UK

Arthritis Research & Therapy 2007, **9(Suppl 3):**P27
(doi: 10.1186/ar2253)

We have previously demonstrated reduced circulating levels of IL-7 in active rheumatoid arthritis (RA) compared with health [1]. Controversy exist as to whether IL-7 correlates or not with C-reactive protein (CRP) in active disease or with other disease activity markers. Normal IL-7 levels were, however, found in 50% of patients in clinical remission and correlated with the recovery of thymic activity [1]. Patients in clinical remission may represent a heterogeneous group, and the aim of this work was to identify predictors of IL-7 recovery in demographic, clinical, imaging and functional data and to compare them with IL-7 in active disease.

One hundred and six patients deemed to be in clinical remission were recruited: stable disease for the preceding 6 months, previous disease duration of at least 12 months, no clinically significant synovitis, CRP below 15 mg/l for the preceding 6 months. Blood and serum were collected from these patients ($n = 106$). Clinical data and imaging data were gathered at the time of sampling. High-sensitivity ELISA was used for cytokine analysis, proliferation assays for T-cell function (mitogen, T-cell receptor stimulation, IL-2, recall antigens); real-time PCR to quantify T-bet and GATA3 expression, and DNA sequencing was carried out to investigate two genetic polymorphisms in the IL-7 gene.

Several studies in healthy controls indicate that normal levels of circulating IL-7 are between 10 and 25 pg/ml. In remission, circulating levels of IL-7 vary between 2.47 and 23.85 pg/ml. Recovery of T-cell function was directly related to levels of circulating IL-7 *in vivo* ($P < 0.001$, $R = 0.873$ for phytohaemagglutinin, $R = 0.786$ for T-cell receptor stimulation and $R = 0.821$ for recall antigen). Age and sex had no effect on IL-7. Combining active ($n = 35$) and remission patients ($n = 106$) showed no indication of a relationship between IL-7 and routine measures of disease activity (CRP, DAS28, erythrocyte sedimentation rate, plasma viscosity and joint counts), suggesting that recovery of IL-7 is not an indicator of remission. To confirm this observation, we used imaging data (MRI and US assessment of hand and wrist) to address whether subclinical disease could predict lack of IL-7 recovery. Evidence of subclinical synovitis was found in 96% of patients in remission [2] despite no evidence of clinically significant synovitis and there was no relationship between IL-7 and imaging scores. However, we found that levels of IL-7 in remission and the age of the patient at disease onset correlate ($P < 0.001$, $R = 0.498$, $n = 86$). A family history of RA and smoking at the time of onset (both self-reported) were also strongly associated with lower levels of IL-7 (both $P < 0.001$, $n = 66$). We analysed two polymorphisms in the promoter and enhancer regions of the IL-7 gene. We found no allele frequency difference between RA patients and healthy controls, remission patients with or without family history, or in relation to IL-7 levels. Finally we analysed circulating cytokines known to regulate IL-7 expression, and found a strong correlation between IL-7 and IFNγ ($P < 0.03$, $R = 0.650$,

$n = 10$). Since IL-7 is a co-activator of Th1 polarisation, we analysed the expression of T-bet and found a direct relationship between the two ($P < 0.01$, $R = 0.601$, $n = 15$) but not with GATA3, which is a regulator of Th2 polarisation.

Recent evidence suggests an important role for IL-7 in the pathogenesis of RA [3]. Our data demonstrate that circulating IL-7 is not an indicator of disease activity. Despite the relationship with age at onset (genetic anticipation) and the family history association, levels of IL-7 in the circulation are apparently not driven by these two polymorphisms. Our results suggest that Th1 polarisation and IL-7 are related and that the mechanism by which IL-7 is recovered in remission may be associated with the recovery of Th1 polarisation.

References

1. Ponchel F, Verburg RJ, Bingham SJ, *et al.*: **Interleukin-7 deficiency in rheumatoid arthritis: consequences for therapy-induced lymphopenia.** *Arthritis Res Ther* 2005, **7:**R80-R92.
2. Brown AK, Quinn MA, Karim Z, *et al.* **Presence of significant synovitis in rheumatoid arthritis patients with disease-modifying antirheumatic drug-induced clinical remission: evidence from an imaging study may explain structural progression.** *Arthritis Rheum* 2006, **54:**3761-3773.
3. Hartgring SAY, Bijlsma JWJ, Lafeber FPJG, van Roon JAG: **Interleukin-7 induced immunopathology in arthritis.** *Annals Rheum Dis* 2006, **65(Suppl 3):**iii69-iii74.

P28

The regulation of IL-7 production in synovial stromal cells in rheumatoid arthritis is promiscuous

S Field, E Jones, A English, C Burgoyne, S Churchman, R Reece, P Emery, F Ponchel

Leeds Institute of Molecular Medicine, Section of Rheumatology, University of Leeds, Leeds, UK

Arthritis Research & Therapy 2007, **9(Suppl 3):**P28
(doi: 10.1186/ar2254)

Background Recent evidence suggests an important role for IL-7 in the pathogenesis of rheumatoid arthritis (RA) and a therapeutic potential for IL-7 blockade [1]. IL-7 is produced by synovial stromal cells (StrC) in RA but is barely detectable in osteoarthritis [2]. Our published data showed reduced production of IL-7 by bone marrow (BM) StrC and reduced levels of circulating IL-7 in RA compared with health [3]. IL-7 was also detected in synovial tissue in active RA but levels were below detection in clinical remission. These conflicting observations suggest that the regulation of IL-7 expression is tightly controlled at the level of tissue specificity. To support this hypothesis, we showed that several cytokines have a different effect on IL-7 production in BM StrC, epithelial cells from the liver and gut. This work aims at identifying the factors that regulate IL-7 production in StrC isolated from synovial tissue of RA patients.

Results Synovial fluid and tissue biopsy were obtained from RA ($n = 6$) and osteoarthritis ($n = 4$) patients (documented with inflammation scores, visual analogue score) and BM aspirates from healthy donors ($n = 6$). Skin fibroblasts were used as negative control. We quantified IL-7 in freshly isolated StC (using an advanced cell-sorting strategy [4]) from healthy BM and showed that IL-7 expression is high. However expression declined rapidly with tissue culture (20-fold by passage 2), suggesting that environmental factors are required to sustain IL-7 expression *in vitro*. IL-7 expression was threefold lower in expanded StrC from RA synovial tissue compared with healthy BM; however, levels were also directly correlated with a visual analogue score of inflammation performed during arthroscopy ($\rho = 0.930$, $P < 0.001$).

IL-7 expression is regulated by several cytokines. IFNγ induced IL-7 by ninefold in health but this regulation is lost in RA in direct relationship with levels of inflammation ($\rho = 0.842$, $P < 0.001$). In contrast, TNFα and IL-1β induced IL-7 by an average of threefold and 15-fold, respectively, in RA but had no effect on healthy BM StrC. The effect of these cytokines was again correlated with exposure to inflammation ($\rho = 0.883$, $P < 0.001$ for TNFα and $\rho = 0.964$, $P < 0.001$ for IL-1β). TGFβ_1 reduced IL-7 expression in health (fivefold) but had no effect in RA. Therefore, the promoter of IL-7 is only sensitive to regulation by other cytokine at low levels of exposure to inflammation. In contrast, IL-7 expression is maximal at high levels of inflammation and cannot be further increased by other cytokines.

Conclusion The expression of IL-7 is tightly controlled in health, the accessibility of the IL-7 promoter to regulation by other cytokines probably being restricted by tissue specificity. In contrast, in RA joints this regulation is abrogated in direct relation with exposure to inflammation. The specific effect of cytokine is therefore only observed when inflammation is low, suggesting promiscuity on the promoter of the IL-7 gene. This may be resulting from an alteration of the IL-7 promoter at the epigenetic level possibly through the effect of Line 1-mediated hypomethylation of chromatin [5].

References

1. Hartgring SAY, Bijlsma JWJ, Lafeber FPJG, van Roon JAG: **Interleukin-7 induced immunopathology in arthritis.** *Annals Rheum Dis* 2006, **65(Suppl 3):**iii69-iii74.
2. Harada S, Yamamura M, Okamoto H, *et al.*: **Production of interleukin-7 and interleukin-15 by fibroblast-like synoviocytes from patients with rheumatoid arthritis.** *Arthritis Rheum* 1999, **42:**1508-1516.
3. Ponchel F, Verburg RJ, Bingham SJ, *et al.*: **Interleukin-7 deficiency in rheumatoid arthritis: consequences for therapy-induced lymphopenia.** *Arthritis Res Ther* 2005, **7:**R80-R92.
4. Jones EA, English A, Kinsey SE, *et al.*: **Optimization of a flow cytometry-based protocol for detection and phenotypic characterization of multipotent mesenchymal stromal cells from human bone marrow.** *Cytom Part B Clin Cytom* 2006, **70:**391-399.
5. Neidhart M, Rethage J, Kuchen S, *et al.*: **Retrotransposable L1 elements expressed in rheumatoid arthritis synovial tissue: Association with genomic DNA hypomethylation and influence on gene expression.** *Arthritis Rheum* 2000, **43:**2634-2647.

P29

Mesenchymal stem cell repair capabilities are defective in rheumatoid arthritis in relation with *in vivo* exposure to inflammation

E Jones, S Field, A English, R Reece, P Emery, D McGonagle, F Ponchel

Leeds Institute of Molecular Medicine, Section of Rheumatology, University of Leeds, Leeds, UK

Arthritis Research & Therapy 2007, **9(Suppl 3):**P29
(doi: 10.1186/ar2255)

Background The potential value of autologous mesenchymal stem cells (MSCs) derived from a patient's joint is now well recognized in osteoarthritis (OA) and rheumatoid arthritis (RA) for the repair of bone and cartilage damage. However, the fact that MSCs have been directly exposed to inflammation in the joint has not been given sufficient attention. We have shown recently that chondrogenesis was qualitatively diminished in MSCs derived from the synovial fluid of RA patients compared with OA [1]. In this report, we investigate the effect of the joint microenvironment on MSCs' repair capability *in vitro* and the possible molecular mechanism by which cells become deficient.

Results Synovial tissue was obtained during arthroscopy from 15 patients with RA and OA, fully documented with visual analogue

scores, which were validated as measure of inflammation locally. High and low levels of inflammation were observed for both diseases. We observed an inverse relationship between levels of inflammation in the joint (VAS) and the chondrogenic potential of MSCs ($R = -0.750$, $P < 0.01$) using an *in vitro* differentiation assay. Such a relationship was not observed for bone differentiation.

The induction of two specific transcription factors (TF) is necessary for differentiation to take place: Sox9 for chondrogenesis and Runx2 for osteogenesis. We measured the levels of expression of these TF in MSCs isolated from nine synovial fluids of RA and OA patients and compared them with MSCs isolated from healthy bone marrow donors ($n = 6$) and skin fibroblasts (negative control, $n = 4$). Sox9 expression was higher in OA than in bone marrow but was reduced to negative control levels in RA. Runx2 expression was reduced in both RA and OA. Sox9 and Runx2 expression was also investigated in synovial tissue MSCs from six RA patients. Sox9 and Runx2 expression were inversely correlated with VAS scores for inflammation ($R = -0.737$, $P < 0.01$ and $R = -0.843$, $P < 0.01$, respectively).

To investigate the effect of proinflammatory cytokines on the ability of MSCs to differentiate we quantified the expression of Sox9 and Runx2 following treatment with TNFα, TGF-β_3, and IFNγ. TGF-β_3 is a trigger of chondrogenesis and accordingly increases the expression of Sox9 and Runx2 in bone marrow MSCs ($n = 4$), whereas TNFα and INFγ reduce the expression of both TF. In RA ($n = 6$), TGF-β_3 had lost the ability to induce the expression of Sox9 and Runx2 whereas TNFα and INFγ increased it. The cytokine effects were all directly correlated with previous exposure to inflammation ($P < 0.05$). Therefore, *in vivo* the joint 'milieu' (which includes these cytokines and several others) is unlikely to allow the induction of a differentiation pathway where TF must be expressed at the right time and amounts and in the right order. Finally, using tissues collected pre and post biologic treatment, we also demonstrated that this deficit can be corrected *in vivo* in a controlled joint milieu (but so far not *in vitro*) – raising the hope that one day cell-based therapies for cartilage repair will be available.

Conclusion Altogether, these data demonstrate that exposure to inflammation has a profound effect on the ability of MSCs to respond to differentiation triggers. Our data also suggest that cytokine(s) are likely to be involved in the acquired deficit in chondrogenesis in RA. Controlling joint inflammation with anti-TNF is associated with the downregulation of inflammation-related parameters (including bone damage [2]) and would therefore be necessary, but may not be sufficient to allow MSCs to undertake cartilage repair.

References

1. Jones EA, English A, Henshaw K, *et al.*: **Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis.** *Arthritis Rheum* 2004, **50:**817-827.
2. Klareskog L, van der Heijde D, de Jager JP, *et al.*: **Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial.** *Lancet* 2004, **363:**675-681.

P30

Diagnostic value of anticyclic citrullinated peptide antibodies in Greek patients with rheumatoid arthritis: association with extra-articular manifestations

Ioannis Alexiou[1], Anastasios Germenis[2], Athanasios Ziogas[1], Athanasios Koutroumbas[1], Katerina Theodoridou[2], Anastasia Kontogianni[1], Lazaros I Sakkas[1]

[1]Department of Rheumatology and [2]Department of Immunology and Histocompatibility, Thessaly University School of Medicine and Hospital, Larisa, Greece

Arthritis Research & Therapy 2007, **9(Suppl 3):**P30
(doi: 10.1186/ar2256)

Background In Northern European Caucasian patients with rheumatoid arthritis (RA), anticyclic citrullinated peptide (anti-CCP) antibodies are associated with the HLA-DRB1 shared epitope and are of diagnostic value. In Greek patients with RA the HLA shared epitope was reported in a minority of patients.

Methods Using an ELISA (CCP2) kit, we tested anti-CCP antibodies in serum samples from 155 Greek patients with RA, 178 patients with other rheumatic diseases and 100 blood donors. We also determined rheumatoid factor (RF) and compared it with anti-CCP antibodies for the area under the curve (AUC), sensitivity, specificity and likelihood ratios. In RA patients we assessed the activity score, and radiographic joint score. Extra-articular manifestations were recorded in an additional 75 RA patients.

Results The sensitivity of anti-CCP antibodies for RA was 63.2% and the specificity was 95.0%. The respective values of RF were 59.1% and 91.2%. When considered simultaneously, the AUC for anti-CCP antibodies was 0.90 with a 95% CI of 0.87–0.93 and the AUC for RF was 0.71 with a 95% CI of 0.64–0.77. The presence of both antibodies increased specificity to 98.2%. Anti-CCP antibodies were positive in 34.9% of RF-negative RA patients. Anti-CCP antibodies exhibited a correlation with the radiographic joint damage (Spearman correlation coefficient $r = 0.27$, $P = 0.001$). Anti-CCP-positive RA patients had increased the swollen joint count and serum CRP concentration compared with anti-CCP-negative RA patients (Mann–Whitney U test, $P = 0.01$ and $P < 0.001$, respectively). However, no correlation was found between anti-CCP antibodies and the DAS28 score ($r = 0.13$, $P = 0.12$). RA patients with high anti-CCP2 antibodies (>100 IU/ml) were more likely to have extra-articular manifestations.

Conclusion In Greek patients with RA, anti-CCP2 antibodies exhibit a better diagnostic value than RF. Anti-CCP2 antibodies show a correlation with joint damage whereas high levels of anti-CCP2 antibodies are associated with extra-articular manifestations. Anti-CCP2 antibodies are therefore useful in rheumatology practice.

P31

Phase 1 study of TRU-015, a CD20-directed small modular immunopharmaceutical (SMIP™) protein therapeutic, in subjects with rheumatoid arthritis

DJ Burge[1], SA Bookbinder[2], AJ Kivitz[3], RM Fleischmann[4], C Shu[5], J Bannink[6], D Barone[6]

[1]Clinical Development, Trubion Pharmaceuticals, Seattle, WA, USA; [2]Clinical Research, Ocala Rheumatology Research Center, Ocala, FL, USA; [3]Clinical Research, Altoona Center for Clinical Research, Duncansville, PA, USA; [4]Department of Rheumatology, Radiant Research, Dallas, TX, USA; [5]Translational Development, Wyeth Research, Collegeville, PA, USA; [6]Research, Trubion Pharmaceuticals, Seattle, WA, USA

Arthritis Research & Therapy 2007, **9(Suppl 3):**P31
(doi: 10.1186/ar2257)

Background CD20-directed therapy is well established in oncology, and was recently demonstrated to be highly effective in the treatment of rheumatoid arthritis (RA) [1]. Small modular immunopharmaceutical drugs are smaller than antibodies yet are capable of utilizing effector functions of antibody-dependent cellular cytotoxicity, complement-mediated cytotoxicity and apoptotic signaling. TRU-015 is a small modular immunopharmaceutical drug candidate that is specific for CD20. TRU-015 has been demonstrated to effectively deplete B lymphocytes in cynomolgus monkeys in a dose-dependent manner, and to improve survival in mouse xenograft tumor models [2,3].

Objectives This phase 1 dose escalation study was performed to evaluate the safety, pharmacokinetics and pharmacodynamics of TRU-015 in subjects with RA.

Methods Thirty-seven RA subjects receiving a stable background of methotrexate were enrolled in eight dosage groups (single dosages of TRU-015 of 0.015, 0.05, 0.15, 0.5, 1.5, 5, or 15 mg/kg, or two doses of 15 mg/kg given 1 week apart (30 mg/kg)). Safety was assessed at baseline, during infusion and at prespecified intervals after infusion by clinical (adverse events, physical examination, vital signs) and laboratory parameters. Serum samples were collected for pharmacokinetic analysis. Pharmacodynamic response was measured using B-cell counts ($CD19^+$ cells by flow cytometry) at prespecified timepoints. Subjects were evaluated for a minimum of 4 weeks and until B-cell recovery.

Results At least four subjects were exposed at each dose level. TRU-015 was generally well tolerated. No dose-limiting toxicities were observed, and no serious adverse events have been reported. Decreases in peripheral B-cell counts were observed in all dose groups at the first time-point after drug administration (24 hours), with doses ≥0.5 mg/kg causing depletion of peripheral B cells. The degree, duration, and recovery of B-cell depletion were dose dependent. Pharmacokinetic parameters of TRU-015 were calculated for cohorts receiving 0.5 mg/kg and higher, and are presented in Table 1. The overall mean half-life of TRU-015 was 295 hours.

Table 1 (abstract P31)

Pharmacokinetics of TRU-015

Group (mg/kg)	Area under the curve from time 0 to infinity (μg/h/ml)	Maximum concentration of drug (μg/ml)	Half-life (hours)	Coefficient of variability (%)
0.5	1,342	13.8	281	59.1
1.5	7,082	58.2	282	26.5
5	18,140	154	295	33.4
15	71,753	462	409	32.8

Conclusion TRU-015, a novel CD20-directed therapy, was generally well tolerated and resulted in dose-dependent B-lymphocyte depletion. Following intravenous infusion, pharmacokinetic properties of TRU-015 (area under the curve, maximum concentration of drug) were approximately dose proportional. Further evaluation of TRU-015 is warranted for the treatment of RA, and other autoimmune diseases.

References

1. Edwards J, Szczepanski L, Szechinski J, *et al.*: **Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis.** N Engl J Med 2004, **350:**2572-2581.
2. Barone D, Baum P, Ledbetter J, Ledbetter MH, Mohler K: **Prolonged depletion of circulating B cells in cynomologus monkeys after a single dose of TRU-015, a novel CD20 directed therapeutic [abstract].** *Ann Rheum Dis* 2005, **64 (Suppl III):**159.
3. Barone D, Nilsson C, Ledbetter J, Hayden-Ledbetter M, Mohler K: **TRU-015, a novel CD20-directed biologic therapy, demonstrates significant anti-tumor activity in human tumor xenograft models [abstract].** *J Clin Oncol ASCO Annual Meeting Proc* 2005, **23(Suppl):**2549.

P32

TRU-015, a small modular immunopharmaceutical (SMIP™) drug candidate directed against CD20, demonstrates clinical improvement in subjects with rheumatoid arthritis

DJ Burge[1], C Shu[2], RW Martin[3], TW Littlejohn[4], DJ Wallace[5], J Taborn[6], WR Palmer[7], A Kivitz[8]

[1]Trubion Pharmaceuticals, Seattle, WA, USA; [2]Wyeth, Collegeville, PA, USA; [3]Arthritis Education & Treatment Center, Grand Rapids, MI, USA; [4]Piedmont Medical Research Association, Winston-Salem, NC, USA; [5]Wallace Rheumatic Study Center, Los Angeles, CA, USA; [6]Midwest Arthritis Center, Kalamazoo, MI, USA; [7]Westroads Medical Group, Omaha, NE, USA; [8]Altoona Center for Clinical Research, Duncansville, PA, USA

Arthritis Research & Therapy 2007, **9(Suppl 3):**P32
(doi: 10.1186/ar2258)

Background Protein therapeutics directed toward CD20 antigen on B lymphocytes have been demonstrated to be highly effective in the treatment of rheumatoid arthritis (RA) [1-3]. Small modular immunopharmaceutical drugs are single-chain polypeptides that are smaller than antibodies. TRU-015 is a CD20-directed small modular immunopharmaceutical drug candidate that effectively depletes B lymphocytes in cynomolgus monkeys in a dose-dependent manner, and improves survival in mouse xenograft tumor models [4,5].

Objective Previously, a dose-escalation study in subjects with RA demonstrated that TRU-015 was generally well tolerated and resulted in dose-dependent B-lymphocyte depletion [6]. The present study was designed to further assess the safety and pharmacokinetics of TRU-015 and to additionally evaluate clinical responses in RA subjects with active disease treated with TRU-015.

Methods Thirty-six RA subjects with active disease despite background methotrexate were enrolled in this randomized, double-blind, multicenter, placebo-controlled study. Subjects were enrolled into one of three cohorts in a 10:2 (active:placebo) ratio to receive TRU-015 as a single intravenous infusion of 5 mg/kg, two infusions of 2.5 mg/kg, or two infusions of 7.5 mg/kg. Subjects in cohorts with two infusions received the infusions 1 week apart. Subjects were premedicated with steroids, but only peri-infusional doses were used.

Results Interim data are available in this ongoing study. TRU-015 was generally well tolerated. No significant infusion reactions were observed. No infectious or noninfectious serious adverse events related to TRU-015 have been reported. B-cell depletion was demonstrated in all cohorts. The exposure of TRU-015 following two intravenous infusion of 2.5 mg/kg was comparable with that following a single infusion of 5 mg/kg. ACR20 responses have been observed in 72% of all evaluable subjects and 82% of rheumatoid factor-positive subjects. At 12 weeks, response rates were similar in each of the cohorts. Longer-term observation is ongoing.

Conclusion TRU-015, administered at doses resulting in B-cell depletion, is generally well tolerated. No significant safety issues have been observed. This study provides evidence that therapy with TRU-015 results in meaningful clinical benefit in subjects with active RA despite methotrexate therapy. Further exploration is required to determine the optimal dose of TRU-015.

References

1. Edwards J, Szczepanski L, Szechinski J, *et al.*: **Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis.** *N Engl J Med* 2004, **350:**2572-2581.
2. Emery P, Fleischmann, R, Filipowicz-Sosnowska A, *et al.*, for DANCER Study Group: **The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results of a phase IIb randomized, double-blind, placebo-controlled, dose-ranging trial.** *Arthritis Rheum* 2006, **54:**1390-1400.
3. Cohen SB, Emery P, Greenwald MW, *et al.*, for the Reflex Trial Group: **Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: results of a multicenter, randomized, double-blind, placebo-controlled, phase II trial evaluating primary efficacy and safety at twenty-four weeks.** *Arthritis Rheum* 2006, **54:**2793-2806.
4. Barone D, Baum P, Ledbetter J, Ledbetter MH, Mohler K: **Prolonged depletion of circulating B-cells in cynomolgus monkeys after a single dose of TRU-015, A novel CD20 directed therapeutic.** *Ann Rheum Dis* 2005, **64:**159.
5. Barone D, Nilsson C, Ledbetter J., Hayden-Ledbetter M, Mohler K: **TRU-015, a novel CD20-directed biologic therapy, demonstrates significant anti-tumor activity in human tumor xenograft models.** *J Clin Oncol ASCO Annual Meeting Proc* 2005, **23(Suppl):**2549.
6. Burge DJ, Bookbinder SA, Kivitz AJ, *et al.*: **Phase 1 study of TRU-015, a CD20 directed small modular immunopharmaceutical (SMIP) protein therapeutic in subjects with rheumatoid arthritis.** *Ann Rheum Dis* 2006, **65(Suppl II):**180.

P33

Quantiferon-TB Gold test in screening for latent tuberculosis before and during antitumour necrosis factor treatment

J Vencovský[1], M Havelková[2], Š Forejtová[1], K Jarošová[1], I Pùtová[1]

[1]Institute of Rheumatology and [2]Mycobacteriology Unit and National Reference Laboratory for Mycobacteria NIPH, Prague, Czech Republic

Arthritis Research & Therapy 2007, **9(Suppl 3):**P33
(doi: 10.1186/ar2259)

Background All candidates for anti-TNF treatment should undergo screening for latent tuberculosis infection (LTBI). BCG vaccination may lead to a positive tuberculin skin test (TST) and false diagnosis of LTBI. Conversely, some immunosuppressed patients may not respond to a TST. Quantiferon TB-Gold (QFT-G) is a new screening tool that uses a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7(p4) proteins to stimulate cells in heparinised whole blood. Detection of IFNγ by ELISA is used to identify *in vitro* responses to these peptide antigens that are specifically associated with *Mycobacterium tuberculosis* infection and do not cross-react with immunity induced by BCG.

Objective To assess the performance of the QFT-G test for screening before the initiation and also during anti-TNF treatment.

Methods The QFT-G test (intube method) was used to determine IFNγ production after stimulation with MT-specific antigens (ESAT-6, CFP-10 and TB7.7(p4) proteins) as well as with nonspecific mitogen (PHA). Altogether, 317 patients were investigated (rheumatoid arthritis (RA) = 117, ankylosing spondylitis (AS) = 95, adult juvenile idiopathic arthritis (JIA) = 54, Crohn's disease (CD) = 30, and psoriatic arthritis (PsA) = 21). Fifteen AS patients completed QFT-G assessments prior to anti-TNF and also after 2 and 14 weeks of treatment (before the second and fourth infliximab infusions). One hundred and eight patients had the QFT-G only before they started anti-TNF, six patients before and during therapy, and 203 patients only during the treatment. Tuberculin 2TU was used for the TST and results were read after 48–72 hours.

Results Out of the total 317 tests, 12 (3.8%) were indeterminate (5 x CD, 4 x RA, 2 x JIA, 1 x AS) (two high spontaneous IFNγ production, 10 low mitogen response). Eleven out of these 12 patients were on combined immunosuppression. Twelve patients (10.5%) (5 x RA, 4 x AS, 2 x CD, 1 x JIA) were positive in the QFT-G in the screening. Four had a negative TST, five a positive, in two the TST was unavailable. From 209 patients investigated during the treatment, 12 were QFT-G-positive. In three of them the initial pretreatment status was known and the QFT-G was negative; however, all three were positive in the TST at 16, 7 and 10 mm. Two of them became QFT-G-positive before the second infliximab infusion, one before the fourth infusion. The remaining nine positive patients had longstanding treatment with different anti-TNF biologicals.

In patients with a positive TST ($n = 64$), 30 had the QFT-G done before treatment – five had positive results and one an indeterminate result. The remaining 34 TST-positive patients were QFT-G-positive in nine cases and one result was indeterminate. In those who were TST-negative ($n = 100$), five were positive for the QFT-G. One patient developed TB, TST-negative, QFT-G-positive before therapy.

In patients who were on infliximab and repeatedly investigated, IFNγ production after the nonspecific mitogen stimulation increased: 6.7 ± 3.8 IU/ml, 8.4 ± 2.6 IU/ml, and 8.9 ± 3.4 IU/ml ($P = 0.001$) before treatment and after 2, and 12 weeks, respectively. No difference was found in IFNγ in longitudinal samples after stimulation with TB antigens.

Conclusion There is scarce information about QFT-G use before and during anti-TNF treatment. Our first experience shows that the QFT-G may be more sensitive and specific for LTBI detection than the TST, although its real usefulness for screening before anti-TNF treatment needs to be assessed in long-term studies. The QFT-G is valuable once anti-TNF has been initiated as we detected a relatively high number of positive patients who may be in danger of TB development. No decrease of the mitogen-induced capacity in IFNγ production shows that test can be meaningfully used during anti-TNF treatment.

Acknowledgement This work was supported by project 0002372801 from the Czech Ministry of Health.

Osteoarthritis

P34

Peroxisome proliferator-activated receptor gamma 1 expression is diminished in human osteoarthritis cartilage and is downregulated by IL-1β in articular chondrocytes

H Afif, L Mfuna, J Martel-Pelletier, J-P Pelletier, H Fahmi
Osteoarthritis Reseach Unit, Centre Hospitalier de l'Université de Montréal, Notre Dame Hospital, Montreal, QC, Canada
Arthritis Research & Therapy 2007, **9(Suppl 3):**P34
(doi: 10.1186/ar2260)

Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor involved in the regulation of many cellular processes. We and others have previously shown that PPARγ activators display anti-inflammatory and chondroprotective properties *in vitro* and improve the clinical course and histopathological features in an experimental animal model of osteoarthritis (OA). However, the expression and regulation of PPARγ expression in cartilage are poorly defined. This study was undertaken to investigate the quantitative expression and distribution of PPARγ in normal and OA cartilage and to evaluate the effect of IL-1β, a prominent cytokine in OA, on PPARγ expression in cultured chondrocytes.
Immunohistochemical analysis revealed that the levels of PPARγ protein expression were significantly lower in OA cartilage when compared with normal cartilage. Using real-time RT-PCR, we demonstrated that $PPAR\gamma_1$ mRNA levels were ~10-fold higher than $PPAR\gamma_2$ mRNA levels; and that only $PPAR\gamma_1$ was differentially expressed, its levels in OA cartilage being 2.4-fold lower than in normal cartilage ($P < 0.001$). IL-1 treatment of OA chondrocytes downregulated $PPAR\gamma_1$ expression in a dose-dependent and time-dependent manner. This effect probably occurred at the transcriptional level, since IL-1 decreases both $PPAR\gamma_1$ mRNA expression and $PPAR\gamma_1$ promoter activity. TNFα, IL-17, and prostaglandin E_2, which are involved in the pathogenesis of OA, also downregulated $PPAR\gamma_1$ expression. Specific inhibitors of the mitogen-activated protein kinase (MAPK) p38 (SB203580) and JNK (SP600125), but not Erk (PD98059), prevented IL-1-induced downregulation of $PPAR\gamma_1$ expression. Similarly, inhibitors of NF-κB signaling (PDTC, MG-132, and SN-50) abolished the suppressive effect of IL-1. In conclusion, our study has demonstrated for the first time that $PPAR\gamma_1$ is downregulated in OA cartilage. The proinflammatory cytokine IL-1 may be responsible for this downregulation via a mechanism involving activation of the MAPKs (p38 and JNK) and NF-κB signaling pathways. The IL-1-induced downregulation of PPARγ expression might be a new and additional important process by which IL-1 promotes articular inflammation and cartilage degradation.

P35

Abnormal collagen type 1 production in subchondral osteoarthritic osteoblasts is responsible in part for altered mineralization in these cells

Denis Chouchourel, Geneviève Bariteau, Aline Delalandre, Daniel Lajeunesse
Unité de recherche en Arthrose, Centre de recherche du CHUM, Hôpital Notre-Dame, Montréal, QC, Canada
Arthritis Research & Therapy 2007, **9(Suppl 3):**P35
(doi: 10.1186/ar2261)

Background Osteoarthritis (OA) is characterized by cartilage damage and loss, synovial membrane inflammation, and bone sclerosis and the formation of osteophytes. Bone sclerosis in OA is due to an abundant osteoid matrix that does not mineralize normally. The mechanism(s) responsible for this abnormal mineralization remain unknown. Here, we studied the link between the mineralization profile of normal and OA osteoblasts (Ob) in primary culture and the mechanisms responsible for this abnormal sclerosis.
Materials and methods We prepared normal and OA Ob from subchondral bone of tibial plateaus. The expression of collagen type 1 α_1 chains (COLL1A1) and α_2 chains (COLL1A2) was determined by real-time PCR. *In vitro* mineralization was evaluated by alizarin red staining. We also determined the mineralization of human SaOS-2 cells, either transfected with the COLL1A1 cDNA or with siRNA for α_1 chains. Conditioned-media (CM) from OA Ob was used to alter the mineralization of SaOS-2 cells while SaOS-2 CM was used to determine whether this could correct OA Ob mineralization.
Results *In vitro* mineralization was reduced in OA Ob compared with normal Ob under basal conditions and following BMP-2 stimulation. This reduced mineralization was accompanied with an increase in COLL1A1 expression in OA Ob compared with normal, with no significant changes in COLL1A2, leading to an elevated COLL1A1 to COLL1A2 ratio in OA Ob. To determine the link between this ratio and mineralization, we used the SaOS-2 cell model. The COLL1A1 to COLL1A2 ratio in SaOS-2 cells varied from 7.5 to 1.5 from day 1 to day 14 postconfluence whereas mineralization progressively increased. Overexpressing COLL1A1 in SaOS-2 cells reduced whereas α_1 chains siRNA transiently increased mineralization. In addition, SaOS-2 CM increased OA Ob mineralization while OA Ob CM reduced mineralization of SaOS-2 cells without any significant changes in the COLL1A1 to COLL1A2 ratio.
Discussion This study suggests that abnormal mineralization of OA bone tissue observed *in vivo* may be linked with an abnormal expression of COLL1A1 and with the release of a putative soluble factor by OA Ob. Production of an abnormal collagen matrix and a soluble factor by OA Ob leads to an abnormal osteoid matrix not mineralizing normally.

P36

Altered expression and production of leptin is responsible for abnormal cell markers in subchondral osteoblasts from osteoarthritic patients

Daniel Lajeunesse, Marie-Solange Mutabaruka, Mohamed Aoulab-Aissa, Aline Delalandre
Unité de recherche en Arthrose, Centre de recherche du CHUM, Hôpital Notre-Dame, Montréal, QC, Canada
Arthritis Research & Therapy 2007, **9(Suppl 3):**P36
(doi: 10.1186/ar2262)

Background Leptin is a peptide hormone with a role in body weight regulation, immune response, bone metabolism and possibly in rheumatic diseases. Osteoarthritis (OA) is characterized by cartilage damage and loss, synovial membrane inflammation and bone sclerosis and the formation of osteophytes. It is now evident that the subchondral bone tissue plays a prominent role in the pathophysiology of OA, a situation that is related to abnormal osteoblast (Ob) differentiation. Leptin can promote the differentiation of Obs; however, a direct role for leptin in human OA has yet to be demonstrated.
Materials and methods We prepared primary cultures of normal and OA Obs from subchondral bone and OA chondrocytes from articular cartilage from tibial plateaus removed for knee replacement surgery of OA patients or at autopsy. We determined the expression and production of leptin using RT-PCR and ELISA.

Alkaline phosphatase activity was determined by *p*-nitrophenyl phosphate hydrolysis, osteocalcin release by enzyme immunoassay and collagen production by the release of the CICP propeptide. $TGF\beta_1$ production was determined by ELISA. Inactivating antibodies raised against leptin receptors and inhibitors of leptin signaling, typhostin (Tyr) and piceatannol (Pce) were used to determine their effect on Ob cell markers. Cellular proliferation was assessed using the BrdU cell proliferation assay whereas activation of the Erk1/2 pathway was determined by western blot analysis.

Results Our results indicated using two different sets of primers for RT-PCR experiments that leptin was expressed only in Obs not in chondrocytes. The expression of leptin was also higher in OA Obs compared with normal using real-time PCR and was responsible for the increase in leptin levels noted in conditioned-media from OA Obs. Although leptin was not expressed by chondrocytes, it was present in articular cartilage – suggesting that leptin produced in bone tissue reached the overlaying cartilage. The long-form leptin receptor mRNA levels were slightly reduced in OA Obs compared with normal Obs. Since leptin can promote the differentiation of Obs, we next questioned whether the observed increase in alkaline phosphatase activity (ALP) and osteocalcin release (OC) observed in OA Obs was linked with their endogenous leptin production. Inactivating antibodies against the leptin receptor reduced both ALP and OC in OA Obs about 35%, a situation reproduced with Tyr and Pce. Likewise, Tyr and Pce also reduced ALP, CICP release, and $TGF\beta_1$ production in OA Obs. Last, leptin dose-dependently (1 ng/ml to 10 mg/ml) stimulated cellular proliferation in OA Obs and this was reflected by an increase in phosphorylation of Erk1/2 signaling.

Discussion These results indicate that OA Obs produce more leptin than normal, and suggest that leptin found in articular cartilage is derived from bone tissue. The increase in leptin in OA bone tissue could also be responsible for increased ALP, OC and $TGF\beta_1$ in Obs. Since leptin can promote inflammation and cartilage loss in combination with cytokines, this suggests that subchondral bone production of leptin may be responsible, at least in part, for cartilage loss in OA.

P37

Molecular mechanisms involved in a differential association of Frzb biology with osteoarthritis and osteoporosis

Rik JU Lories, Jan Schrooten, Inge Derese, Jenny Peeters, Astrid Bakker, Frank P Luyten

Department of Musculoskeletal Sciences, Division of Rheumatology, Katholieke Universiteit Leuven, Belgium

Arthritis Research & Therapy 2007, **9(Suppl 3):**P37
(doi: 10.1186/ar2263)

Background Nonsynonymous polymorphisms in the human FRZB gene have been associated with osteoarthritis (OA). In addition, a differential association between OA and osteoporosis (OP) has been reported. We have demonstrated that genetic deletion of the Frzb gene in mice increases cartilage damage in different models of OA. In addition, $Frzb^{-/-}$ mice show increased cortical bone density.

Objective To study the underlying molecular mechanisms involved in cartilage damage and increased bone density in $Frzb^{-/-}$ mice.

Methods Active Wnt signaling in the articular cartilage was studied in normal and methylated bovine serum albumin, collagenase and papain-induced arthritis using β-catenin (CTNNB1) immunohistochemistry. Gene expression patterns of components of the Wnt signaling pathway, its antagonist and target genes, were studied using multigene cDNA arrays (Superarray) in microdissected articular cartilage and soft tissues of healthy and affected knees from $Frzb^{-/-}$ and wild-type mice. Gene expression of tissue destructive enzymes in the cartilage was studied by real-time PCR. Matrix metalloproteinase-3 activity was tested *in vitro*. Cortical bone stiffness was estimated by compression of the ulnae. Mechanical loading-induced bone adaptation was studied by compression of the ulnae of 17-week-old mice, followed by microcomputed tomography analysis at a resolution of 5 ìm. Subchondral bone properties were studied with histomorphometry, peripheral quantitative computed tomography and microcomputed tomography.

Results In healthy cartilage of $Frzb^{-/-}$ mice, four genes were consistently expressed at lower levels than in the wild-type mice: Ctnnb1, Ctbp1, Fosl1 and Myc. In contrast, Wnt8b expression was upregulated in healthy cartilage in 2/3 samples from $Frzb^{-/-}$ versus wild-type mice and in all samples in affected versus healthy wild-type cartilage. Ctbp1 and Fosl1 were also downregulated in all samples from arthritic wild-type mice versus healthy ones. In addition, Wnt8b appears to be downregulated in arthritic versus healthy joints of $Frzb^{-/-}$ mice, suggesting a distinct regulation of Wnt ligand expression in the genetic model as compared with the wild-type mice. Cartilage damage in $Frzb^{-/-}$ mice is associated with increased canonical Wnt signaling, matrix metalloproteinase-3 expression and activity. In addition, the $Frzb^{-/-}$ mice have an increased cortical bone thickness and density, resulting in stiffer bones as demonstrated by a different stress–strain relationship in $Frzb^{-/-}$ mice. Moreover, the periosteal anabolic response to mechanical loading is significantly greater in $Frzb^{-/-}$ mice than in wild-type mice. FRZB is expressed in the periosteum.

Conclusion The preinduction gene expression profile in $Frzb^{-/-}$ mice shows similarities with arthritic cartilage in wild-type mice. Loss of Frzb may contribute to cartilage damage by increased expression and activity of matrix metalloproteinase in both a Wnt-dependent and Wnt-independent manner. FRZB deficiency also results in thicker cortical bone with increased stiffness and higher cortical appositional bone formation after loading. This may contribute to OA by producing increased strain on the articular cartilage during locomotion. Increased cortical density of long bones in $Frzb^{-/-}$ mice supports our earlier observation that polymorphisms in the human FRZB gene are differentially associated with hip OA and OP hip fractures. The role of FRZB in cartilage and bone biology may therefore provide a mechanistic basis for the longstanding clinical observation that OA and OP show an inverse relationship.

P38

Matrix remodeling during enchondral ossification and aggrecan cleavage in osteoarthritic cartilage depends on syndecan-4

Frank Echtermeyer[1], Katja Neugebauer[1], Ingmar Meinecke[1,2], Christine Herzog[3], Rita Dreier[4], Thomas Pap[1]

[1]Division of Molecular Medicine of Musculoskeletal Tissue, [2]Department of Traumatology, [3]Department of Anatomy and Anaesthesiology, and [4]Department of Physical Chemistry and Pathobiochemistry, University Hospital Munster, Germany

Arthritis Research & Therapy 2007, **9(Suppl 3):**P38
(doi: 10.1186/ar2264)

Chondrocyte differentiation into hypertrophic chondrocytes is essential for enchondral ossification of long bones during limb development, but plays a role also in pathologic situations such as osteoarthritis (OA). However, the mechanisms that link chondrocyte hypertrophy to cartilage remodeling are poorly understood. Based on recent data that have implicated transmembrane

heparan sulfate proteoglycans in matrix turnover and cell differentiation, we analyzed the role of syndecan-4 during limb development in mice and studied its expression and function in OA.

Syndecan-4 promoter activity was detected in whole embryos by staining for β-galactosidase in syndecan-4$^{-/-}$ LacZ knockin mice. For cellular localization of syndecan-4 expression within cartilage, immunohistochemistry with antibodies against syndecan-4 and type X collagen was performed. Alizarin red S staining was carried out to analyze the mineralization of bones in wild-type and syndecan-4$^{-/-}$ littermates at days E13.5 and E14.5. To study syndecan-4 in OA, we compared its expression in normal and OA articular cartilage by Northern blot analysis and immunohistochemistry. Blocking antibodies against syndecan-4 were generated and used to analyze the role of syndecan-4 in IL-1-mediated proteoglycan loss *in vitro*. For functional analysis *in vivo*, osteoarthritic changes were induced in syndecan-4$^{-/-}$ mice and in wild-type controls by surgically achieved joint instability, and the loss of proteoglycans was assessed by safranin-orange staining. Staining for syndecan-4 and ADAMTS-generated aggrecan neo-epitopes was performed in the knees of these mice.

β-Galactosidase-staining of syndecan-4$^{-/-}$ mice at E12.0 showed a strong activity of the syndecan-4 promoter at sites of cartilage condensations. In later stages, syndecan-4 was detected in the growth plates of long bones. In wild-type embryos, syndecan-4 protein was also found mainly in chondrocytes of the hypertrophic zone, where it colocalized with type X collagen. The loss of syndecan-4 was associated with a significant retardation in the mineralization of axial and appendicular bones. Interestingly, there was a clear upregulation of syndecan-4 in human OA cartilage both at the mRNA and the protein level. In the cartilage explant model, blocking antibodies to syndecan-4 substantially significantly reduced the IL-1-induced loss of proteoglycans in wild-type cartilage. Analysis of OA-like changes in mice revealed a strong and early induction of syndecan-4, and there was a significant reduction of proteoglycan loss in the syndecan-4$^{-/-}$ mice compared with their wild-type controls. This was accompanied by a significantly reduced staining for ADAMTS-generated aggrecan neo-epitopes in syndecan-4$^{-/-}$ mice.

Our data show that syndecan-4 is induced in hypertrophic chondrocytes both during embryogenesis and in OA cartilage. By promoting ADAMTS-mediated cleavage of aggrecans, syndecan-4 facilitates enchondral ossification but is involved also in cartilage degradation by hypertrophic chondrocytes in OA. Inhibition of syndecan-4 may therefore constitute a promising strategy to interfere with osteoarthritic cartilage damage.

P39

Differential Toll-like receptor-dependent collagenase expression in chondrocytes

Qian Zhang[1], Wang Hui[1], Rose Davidson[2], Clare Darrah[3], Simon T Donell[3], Ian M Clark[2], Tim E Cawston[1], John H Robinson[1], Andrew D Rowan[1], David A Young[1]

[1]Newcastle University, Newcastle-upon-Tyne, UK; [2]University of East Anglia, Norwich, UK; [3]Norfolk & Norwich University Hospital, Norwich, UK

Arthritis Research & Therapy 2007, **9(Suppl 3):**P39
(doi: 10.1186/ar2265)

Objective To characterise the catabolic response of osteoarthritic (OA) chondrocytes to ligands that activate the complete repertoire of Toll-like receptors (TLRs).

Methods The induction of MMP-1 and MMP-13, the collagenases, by ligands that activate the complete repertoire of TLRs was assessed in OA chondrocytes by quantitative RT-PCR. TLR ligand signalling pathway activation and their role in collagenase induction was analysed by western blotting and selective pathway inhibitors and siRNA. TLR ligand expression was compared in total RNA from femoral head cartilage of normal (neck of femur fracture) and OA patients undergoing joint replacement surgery.

Results All the ligands upregulated MMP-1 and MMP-13, although to differing degrees, indicating differential regulation of the collagenases and indicating that chondrocytes express most TLRs. MALP-2 (TLR6/2 ligand) and poly(IC) (a dsRNA mimicking TLR3 ligand) most robustly upregulated MMP-1 and MMP-13, respectively. Using siRNA the activation of MMP-13 by TLR3 and TLR6/2 ligands were confirmed to be via the adaptor Trif and MyD88. The induction of both MMP-1 and MMP-13 by TLR1/2, TLR3 and TLR6/2 ligands were dependent upon the NF-κB pathway. Interestingly, MMP-1 and MMP-13 induction by the three TLR ligands were differentially inhibited by various MAPK inhibitors, with MMP-13 induction sensitive to ERK pathway inhibition. In a cartilage resorption assay, TLR1/2 and TLR6/2 ligands, but not TLR3 ligand, led to significant collagen release. The expression profile of TLRs in neck of femur and OA cartilage revealed a highly significant ($P < 0.001$) downregulation of TLR2 and upregulation of TLR3.

Conclusion Our gene expression profile reveals that chondrocytes express a large repertoire of TLRs, which upon activation leads to collagenase gene activation. The TLR1/2 and TLR6/2 ligands, which resulted in cartilage collagen resorption, require TLR2, whose expression is significantly repressed in end-stage OA. These data suggest modulation of TLR-mediated signalling, particularly via TLR2, as a potential therapeutic strategy for OA prevention.