SAT0028

THE IMPORTANCE OF PROPER HANDLING OF HUMAN SYNOVIAL FLUID FOR ARTHRITIS RESEARCH

H. Brouwers¹, J.H. von Hegedus¹, R.E.M. Toes¹, T.W.J. Huizinga¹, M.A. Giera², M. Kloppenburg¹, A. Ioan-Facsinay¹. ¹Rheumatology; ²Center for Proteomics and Metabolomics, Leiden University medical Center, Leiden, Netherlands

Background: Synovial fluid (SF) is commonly used for diagnostic and research purposes since it is easily obtained and is thought to represent the local inflammatory environment. Common practice to obtain insight in the disease pathogenesis of arthritic diseases is the characterisation and quantification of synovial fluid cells and the measurement of inflammatory mediators. SF has a complex composition, containing hyaluronic acid which makes the fluid viscous and non-homogeneous, hampering subsequent analyses. Hyaluronic acid can be broken down by the addition of hyaluronidase, however, this is not commonly used in arthritic research.

Objectives: To determine the effect of hyaluronidase treatment on quantification and identification of SF cells and soluble mediators.

Methods: SF was obtained from twelve arthritis patients after knee aspirations as part of standard clinical care. Nine patients were diagnosed with rheumatoid arthritis, two with osteoarthritis and one with juvenile idiopathic arthritis. For cell analysis, synovial fluid was first centrifuged and the pellet was separated from the fluid. The fluid was subsequently treated with hyaluronidase and centrifuged again to isolate remaining cells. Cell numbers and phenotype were determined using flow cytometry. For soluble mediator measurements, 6–10 aliquots were taken and treated as represented in figure 1 resulting in set 1 and set 2. Set 1 contains replicates taken from SF before hyaluronidase treatment while set 2 mimics replicates which are taken after hyaluronidase treatment. Interleukin (IL)–8 was measured by ELISA and a total of seven fatty acid and oxidised fatty acid levels were determined using LC-MS/MS in all aliquots.

Results: Between 0.8%–70% of immune cells (median 5%) are lost when the SF is not treated with hyaluronidase. This percentage is higher for T and B cells: 7%–85% (median 22%) and 7%–88% (median 23%), respectively. To assess the variation between the soluble mediator concentrations in set 1 and set 2, the coefficients of variation (CV) of the replicate measurements were compared. Aliquots in set 2 showed a lower CV for the oxidised lipids 17-HDHA, leukotriene B₄ and prostaglandin E₂ for all patients tested. For IL-8, the oxidised lipid 15-HETE, and fatty acids arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), it did not matter whether the aliquots were taken before or after hyaluronidase treatment. While in general the average levels of the soluble mediators tested were comparable between set 1 and set 2, we did observe large differences in IL-8 and AA levels in two independent patients.

Conclusions: Up to 70% of the synovial fluid cells are missed in analysis when the SF is not treated with hyaluronidase before cell isolation, leading to erroneous conclusion especially when investigating rare cells populations like antigen specific B or T cells, when searching for novel cell populations, or when correlations are made between clinical parameters and cell numbers. In addition, some cytokine as well as lipid levels determined in SF without hyaluronidase treatment might not accurately reflect their actual concentrations in SF.

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SAT0029

IMPACT OF GLUCOCORTICOIDS ON SIRTUIN 1 EXPRESSION AND PROINFLAMMATORY CYTOKINE PRODUCTION IN RATS WITH ADJUVANT-INDUCED ARTHRITIS

D. Wendling¹, W. Abbas², A. Kumar², S. Pasquereau², F. Verhoeven¹, C. Prati¹, C. Demougeot³, G. Herbein². ¹Rheumatology, CHRU Besançon; ²EA4266 EPILAB; ³EA4267 Pepite, University BFC, Besançon, France

Background: Sirtuin 1 (SIRT1) is a class III histone deacetylase which could play a critical role in several biological functions including lifespan, stress, and inflammation.

Objectives: Our main objective was to evaluate SIRT1 expression in peripheral blood mononuclear cells (PBMCs) isolated from rats with adjuvant induced arthritis (AIA) treated or not with low and high doses of glucocorticoids (GCs). Our second aim was to determine the production of proinflammatory cytokines such as tumour necrosis factor alpha (TNF), interleukin-1 beta (IL-1) and interleukin-6 (IL-6) in tissues (liver and spleen) of these rats, and to assess a potential correlation between SIRT1 expression and the production of cytokines in tissues of rats with AIA treated or not with low and high doses of glucocorticoids.

Methods: We performed a study on rats with AIA treated with low and high doses of prednisolone or left untreated. The SIRT1 expression was quantified in PBMCs using western blotting. Levels of proinflammatory cytokines TNF, IL-1 and IL-6 were assessed in liver and spleen from rats with AIA treated with GCs or left untreated using an ELISA assay.

Results: SIRT1 expression was increased in PBMCs isolated from rats with AIA treated with LD-GCs and HD-GCs compared to untreated animals. In culture, resveratrol enhanced the SIRT1 expression in PBMCs isolated from rats with AIA in a dose-dependent manner. We observed increased levels of TNF alpha in liver (correlated with SIRT1 expression) and decreased IL-6 levels in spleens (negatively correlated with SIRT1 expression) from rats with AIA treated with LD-GCs and HD-GCs compared to controls.

Conclusions: SIRT1 expression is increased in PBMCs isolated from rats with AIA and treated with GCs parallel to decreased levels of IL-6 in spleen and increased levels of TNF in liver. This study shows that the SIRT 1 expression is measurable in circulating PBMCs of rats with AIA and is increased under GC treatment. The potential epigenetic effect of GCs on production of proinflammatory cytokines in tissues, namely liver and spleen, is also studied.

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SAT0030

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

D.M. Küppers¹, L. Tsiklauri¹, M.-L. Hülser¹, K. Frommer¹, S. Rehart², U. Müller-Ladner¹, E. Neumann¹. ¹Dept. of Rheumatology and Clinical Immunology, Justus-Liebig-University Gießen, Campus Kerckhoff, Giessen; ²Dept. of Orthopaedics and Trauma Surgery, Agaplesion Markus Hospital, Frankfurt/Main, Germany

Background: Destructive bone diseases like osteoarthritis and osteoporosis are causes of chronic pain and disability. Mesenchymal stromal cells (MSC) are key regulators for bone homeostasis due to their potential for osteogenic differentiation, preventing bone loss and promoting tissue repair. Pro-inflammatory factors including adipokines such as visfatin alter the osteogenic potential of MSCs and may contribute to the shift towards adipogenic differentiation observed in osteoporosis. Long non-coding RNA H19 is one of the first lncRNAs discovered and best understood e.g. in embryonic growth and tumour formation. lncRNAs interact directly with DNA, RNA as well as proteins and fill a regulatory gap between the ribonucleic and protein world. H19 upregulation was shown in osteogenic differentiation of MSCs¹. H19 increases the osteogenic potential by acting as a sponge for miRNA 675, 141 and 22, influencing TGFβ1 and Wnt/β-catenin pathways^{2,3}. H19 may hence be involved in MSC mediated bone homeostasis.

Objectives: To analyse the link between adipokines and bone remodelling in destructive bone disease. To investigate the influence of adipokines on MSCs and on the expression of lncRNA H19 during osteogenesis.

Methods: Commercial human MSCs (hMSC) and primary human MSCs (phMSC) from osteoarthritis patients after knee replacement surgery were treated with differentiation medium to induce osteogenic differentiation (OD). Matrix mineralization was quantified after 21 days of OD by Alizarin Red. Expression of H19

and IL-6 protein production were measured using realtime PCR and ELISA at day 2/7/14 of OD.

Results: H19 up-regulation in MSCs during OD could be confirmed in a time dependent manner. Visfatin-stimulation of MSC during OD increased matrix mineralization over time as well as IL-6 production (day 7, 14, 21: 46-, 93-, 78-fold). Visfatin stimulation down-regulated H19 expression up to 10-fold over the course of OD compared to non-stimulated control. The effect was significant in phMSCs in 2/3 measured time points (day 7, $p=0.03$; day 14, $p=0.002$, $n=3$) and in hMSCs on day 14 ($p=0.0003$, $n=4$).

Conclusions: During osteogenic differentiation of MSCs, visfatin showed pro-inflammatory and mineralization promoting effects. However, H19 was significantly down-regulated by visfatin during osteogenic differentiation. This may contribute to the loss of osteogenic potency of MSCs in inflamed tissues with increased visfatin concentration as observed in affected areas of destructive bone disease. Further research to understand the H19 effector mechanisms on osteogenic differentiation and osteogenic potency of MSCs are in progress.

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SAT0031 INTERLEUKIN 29 INHIBITS OSTEOCLAST DIFFERENTIATION AND FUNCTION IN RANKL-INDUCED OSTEOCLASTOGENESIS

F. Wang, A. Luo, Q. Wu, Z. Zhou, Q. Peng, W. Xuan, W. Tan. *The First Affiliated Hospital of Nanjing Medical University, Nanjing, China*

Background: Interleukin-29 (IL-29) is a new member of the recently discovered interferon (IFN) λ family and known to modulate immune functions of monocyte or macrophage. We have demonstrated that IL-29 is dysregulated in patients with rheumatoid arthritis (RA) and contributes to RA pathogenesis via inducing proinflammatory cytokines, chemokines or matrix metalloproteinases production in synovial fibroblasts,^{1,2} as well as stimulating inflammation and cartilage degradation in osteoarthritis disease.³ However, the role of IL-29 in bone resorption is unclear.

Objectives: Bone erosion in RA is associated with increased production of pro-inflammatory cytokines and accelerated osteoclastogenesis in affected joints.⁴ IL-29 is an important proinflammatory cytokine in RA. We investigated the effect of IL-29 on receptor activator of nuclear factor κ B ligand (RANKL)-induced osteoclastogenesis *in vitro* to determine whether IL-29 can stimulate or attenuate osteoclast-mediated bone resorption that is a hallmark of RA.

Methods: The viability and apoptosis of RAW264.7 cells after IL-29 different treatment were assessed by Cell Counting Kit-8 and flow cytometry, respectively. Osteoclasts were generated from RAW264.7 cells, bone-marrow-derived monocyte/macrophage precursor cells (BMMs) and blood-derived human PBMC cells. The effects of IL-29 on osteoclast formation were evaluated by tartrate-resistant acid phosphatase (TRAP) staining and counting the number of TRAP + multinucleated cells. Bone resorption experiment was assessed by pit formation. The expression of key molecules implicated in osteoclastogenesis (TRAP, CTSK, MMP-9, NFATC1 and c-Fos) was measured by real time RT-PCR.

Results: Although IL-29 did not show significant effect on the viability and apoptosis of RAW264.7 cells, it inhibited multinucleated cells in the differentiation of murine and human osteoclastogenesis, the bone-resorbing activity of mature osteoclasts. In addition, IL-29 downregulated osteoclast specific genes expression of TRAP, CTSK, MMP-9, NFATC1 and c-Fos.

Conclusions: IL-29 inhibits murine and human osteoclastogenesis by a direct mechanism suppressing responses of osteoclast precursors to RANKL. Our findings suggest that IL-29 may play a previously unrecognised role in the osteoclast formation.

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SAT0032 MESENCHYMAL STEM CELLS ALLEVIATE EXPERIMENTAL AUTOIMMUNE CHOLANGITIS THROUGH IMMUNOSUPPRESSION MEDIATED BY GALECTIN-9

J. Fan, X. Tang, H. Chen, L. Sun, Q. Wang. *Department of Rheumatology and Immunology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, nanjing, China*

Background: Mesenchymal stem cells (MSCs) play anti-inflammatory role by secreting some kinds of bioactive molecules. However, the effect of MSCs on chronic autoimmune liver disease, such as primary biliary cholangitis (PBC) and its underlying mechanism remains elusive.

Objectives: The aim of this study was to assess the efficacy of UC-MSCs treatment (UC-MSCT) in 2OA-BSA-induced murine autoimmune cholangitis and explore its underlying mechanisms.

Methods: UC-MSCs were transplanted into experimental autoimmune cholangitis mice. Biochemical and histological analysis were performed based on the blood and liver tissues. The immunomodulatory effects of UC-MSCs and its cytoprotective function were also investigated.

Results: We found that UC-MSCT significantly ameliorated liver inflammation in 2OA-BSA induced autoimmune cholangitis mice, primarily by diminishing Th1 and Th17 responses, and modifying liver chemokine activity. We also found that UC-MSCs significantly repressed the proliferation of CD4⁺T cells and suppressed the differentiation of Th1 and Th17 cells, both of which were dependent on galectin-9 (Gal-9). Furthermore, we determined the signal transducer and activator of transcription (STAT) and c-Jun N-terminal kinase (JNK) signalling pathways were involved in the production of Gal-9 in MSCs.

Conclusions: The present study shows that UC-MSCs exert profound inhibitory effects on inflammatory responses and that they ultimately alleviate the liver injury in experimental autoimmune cholangitis mice. Further, we demonstrate that UC-MSCs inhibit Th1 and Th17 cell responses as well as aberrant chemokine activity through Gal-9 mediated immunosuppression. Additionally, our research reveals that the induction of Gal-9 in MSCs is mediated by the involvement of the STAT and JNK signalling pathways. These findings may help in the development of stem cell therapies for the treatment of PBC.

Disclosure of Interest: None declared

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SAT0033 TIME-DEPENDENT RELATIONSHIPS BETWEEN BIOLOGICAL PARAMETERS AND DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

K. Connelly¹, H. Nim², F. Vincent¹, F. Petitjean², A. Hoi¹, R. Kohlmeyer¹, S. Boyd², E. Morand¹. ¹Centre for Inflammatory Diseases; ²Faculty of Information Technology, Monash University, Clayton, Australia

Background: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterised by high inter-patient variability of clinical features, pathology, and disease time-course. Relationships between biomarkers and disease remission/relapse cycles are especially complex and poorly understood.

Objectives: To investigate the relationship between disease activity and biomarker expression in a longitudinally-followed SLE cohort.

Methods: We measured 4 candidate protein biomarkers implicated in SLE (MIF, CCL2, CCL19 and CXCL10) and 13 routinely collected serum and urine biological parameters, and assessed disease activity (SLEDAI-2k) on each clinic visit. We analysed these data by first focusing on the magnitude of expression levels of the 17 biological markers and then on the temporal dimension, to untangle their relationship to disease activity.

Results: Data from 843 visits in 110 SLE patients (median age 47, 83% female, 49% Asian ethnicity) were analysed. We demonstrated highly heterogeneous time-dependent relationships between disease activity and the measured