

References:

- [1] Pérez-García S, Gutiérrez-Cañas I, Seoane IV, et al. *Am J Pathol.* 2016; 186(9):2449–61.
- [2] Yuasa T, Otani T, Koike T, et al. *Laboratory Investigation.* 2008;88(3):264–74.
- [3] Luyten FP, Tylzanowski P, Lories RJ. *Bone.* 2009;44(4):522–7.
- [4] Ji Q, Xu X, Xu Y, Fan Z, Kang L, Li L, et al. *J Mol Med (Berl).* 2016;94(6):681–94.
- [5] Komori T. *Cell and tissue research.* 2010;339(1):189–95.

Acknowledgements: This work has been supported by Instituto de Salud Carlos III, Spain, cofinanced by FEDER, European Union: RETICS program, Red de Investigación en Inflamación y Enfermedades Reumáticas (RD16/0012/0008 (RPG) and RD16/0012/0011 (IGA) and the projects PI12/00758 (RPG), PI14/00477 (CMM) and PI14/00442 (IGA).

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5808

FRI0017 A NOVEL CONCEPT OF M1 AND M2 MONOCYTES IN RHEUMATOID ARTHRITIS: PRO-INFLAMMATORY MONOCYTE POLARIZATION IMBALANCE, ANTI-CITRULLINATED PROTEIN ANTIBODY AND OSTEOCLASTOGENESIS

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Background: Monocytes can differentiate into either proinflammatory, microbicidal M1 macrophage or anti-inflammatory M2 macrophage subtypes. In addition to macrophages, regarding monocyte subsets, M1 monocytes and M2 monocytes mirroring the M1/M2 macrophage polarization concept were suggested. Little is known regarding the relationships between osteoclastogenesis and M1/M2 monocyte subsets.

Objectives: We investigated the relationships among M1 monocytes, M2 monocytes, osteoclast differentiation ability and clinical characteristics in patients with rheumatoid arthritis (RA).

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from RA patients and healthy donors, and we then investigated the number of M1 monocytes or M2 monocytes by fluorescence-activated cell sorting. We defined positive CD14, CD68 and CCR2 monocytes as M1 monocytes, and in separate tubes, we defined positive CD14, CX3CR1 and CD163 or CD206 monocytes as M2 monocytes.

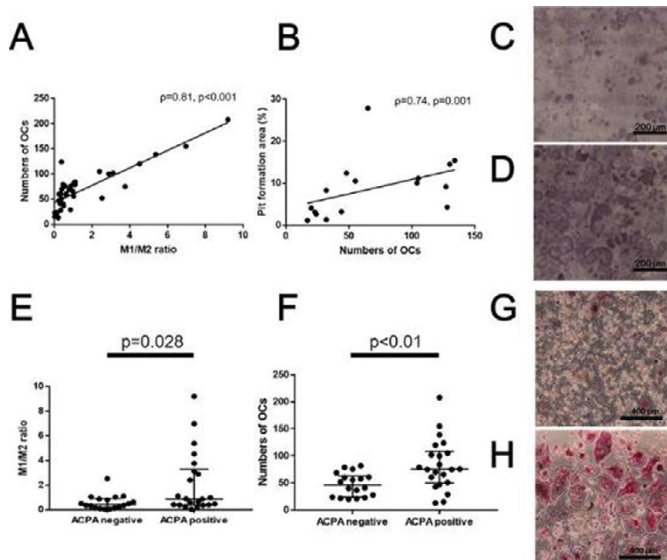
We also obtained and cultured CD14-positive cells from PBMCs from RA patients and healthy donors to investigate osteoclast differentiation in vitro. We defined osteoclasts as tartrate-resistant acid phosphatase (TRAP) staining-positive cells with >3 nuclei. We counted the osteoclasts in the whole wells of a 96-well dish. Pit formation assays were performed to evaluate function of osteoclasts.

Results: Forty RA patients and 20 healthy donors were included. Twenty-two patients (55%) were ACPA-positive.

The median M1/M2 ratio was 0.59 (0.31–1.11, IQR). There were no significant differences between the RA patients and healthy donors.

There was a positive correlation between the M1/M2 ratio and the differentiated osteoclast number in vitro in RA patients ($\rho=0.81$, $p<0.01$) (A). The numbers of osteoclasts in vitro were significantly correlated with the area percentage of the pit formation area ($\rho=0.74$, $p=0.001$) (B). We demonstrated that the RA patients who had lower M1/M2 ratio and fewer osteoclasts (C) had smaller resorbed areas compared to the RA patients who had higher M1/2 ratio and greater numbers of osteoclasts (D).

The ACPA-positive patients had significantly higher M1/M2 ratio in vivo ($p=0.028$)



(E) and significantly greater numbers of osteoclasts in vitro ($p<0.01$) (F) than the ACPA-negative patients. We show an ACPA-negative patient's osteoclasts in vitro (G) and those of an ACPA-positive patient (H).

Multivariable regression analysis revealed that the M1/M2 ratio was the sole significant contribution factor to in vitro osteoclastogenesis (β -coefficient 16.3, $p<0.0001$).

RA patients with M1/M2 ratio >1 (having relatively more M1 monocytes) had higher erythrocyte sedimentation rates ($p=0.011$) and C-reactive protein ($p=0.032$) than RA patients with M1/M2 ratio ≤ 1 .

M1-dominant monocytes in vitro produced higher concentrations of IL-6 upon stimulation with lipopolysaccharide than M2 monocytes ($p=0.032$).

Conclusions: The M1/M2 ratio is strongly correlated with the in vitro differentiation of osteoclasts in patients with RA. The RA patients with positive ACPA had higher M1/M2 ratio and higher numbers of osteoclasts.

M1 and M2 monocyte subsets may become a new target of treatments for RA.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2083

FRI0018 TARGETED INHIBITION OF JANUS KINASES ABATES IFN-GAMMA-INDUCED INVASIVE BEHAVIOR OF FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Emerging evidence suggests that fibroblast-like synoviocytes (FLS) are key effector cells in rheumatoid arthritis (RA) and research into the mechanisms defining FLS activity in RA indicate that cytokines secreted by leukocytes play a crucial role. Nevertheless, the contribution of IFN γ , which is increased in rheumatoid synovitis, to the inflammatory synovial tissue reaction is not known.

Objectives: To explore the function of the T-cell cytokine IFN γ for mesenchymal tissue remodeling in RA, and to determine whether IFN γ -signaling controls the invasive potential of FLS.

Methods: To assess architectural responses, FLS were cultured in three-dimensional micromasses. FLS motility was analyzed in migration-, spreading- and invasion assays. Signaling events relevant to cellular motility were defined by western blots. Baricitinib and siRNA pools were used to suppress Janus Kinase (JAK) functions.

Results: Histological analyses of micromasses revealed unique effects of IFN γ on FLS shape and tissue organization. This was consistent with accelerated migration, pronounced actin and focal adhesion (FA) re-organization upon IFN γ stimulation. Since actin and FA dynamics and, thus, cell motility are integrated by the focal adhesion kinase (FAK), we next analyzed its activity. Indeed, IFN γ stimulation induced the phosphorylation of FAK-Y925, a phosphosite implicated in FAK-mediated cell migration. siRNA knockdown of JAK2, but not JAK1, abrogated FAK activation by IFN γ . Correspondingly, IFN γ -induced FAK activation and invasion of FLS was abrogated by the JAK-inhibitor baricitinib.

Conclusions: Our study contributes insight into the synovial response to IFN γ and reveals JAK2 as a potential therapeutic target for FLS-mediated joint destruction in arthritis, especially in RA.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4862

FRI0019 HUMAN MESENCHYMAL STROMAL CELLS REDUCE TNF α SECRETION OF ACTIVATED PBMC VIA CTLA-4

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Background: The inhibitory costimulatory molecule Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) plays a crucial role in conveying immune tolerance in both inflammatory disorders and regenerative processes such as bone healing. During regenerative processes, mesenchymal stromal cells (MSC) provide the building bricks for reestablishing structural integrity but do also control inflammation by their immunomodulatory activities under restrictive microenvironmental conditions such as hypoxia. Here, we hypothesize these cells to support the control of inflammation via CTLA-4 in order to facilitate tissue regeneration such as bone fracture healing.

Objectives: Therefore, we analyzed expression of CTLA-4 by human MSC and their ability to convey immune suppression.

Methods: MSC were isolated from bone marrow of patients undergoing total hip replacement and characterized (i) by surface marker staining using flow cytometry and (ii) by assessing their osteogenic and adipogenic differentiation potential. MSC were cultured under normoxic (~18% O $_2$) and hypoxic (<1.5% O $_2$) con-

ditions, respectively. CTLA-4 expression in MSC was analyzed on protein and on transcriptional levels by flow cytometry, immunoblotting, ELISA and by quantitative PCR, respectively. To assess the functionality of CTLA-4 expression, MSC were co-cultured with peripheral blood mononuclear cells (PBMC) isolated by density gradient centrifugation from venous blood of healthy donors. The cells were stimulated for 48h with PHA (5 μ g/ml) or left untreated and challenged using either CTLA-4-Ig or anti-CTLA-4-antibody, incubated under normoxic (~18% O₂) or hypoxic (<1.5% O₂) conditions and analyzed for TNF α secretion by suspension assay.

Results: The MSC phenotype of bone-marrow derived cells could be verified according to their surface marker expression and their osteogenic and adipogenic differentiation capacity. On transcriptional level, MSC express both the full-length and - to a higher extent - the soluble CTLA-4 isoforms with a higher mRNA abundance under normoxic as compared to hypoxic conditions. Extra- and intracellular analysis of CTLA-4 expression on protein level, demonstrated a significant shift of the whole MSC population (p<0.01). CTLA-4 expression and secretion by MSC was confirmed using immunoblot and ELISA, respectively. Co-culture of MSC with PHA-activated PBMC significantly reduced the amount of secreted TNF α (p<0.05) which could be reversed by anti-CTLA-4-antibody (p<0.05), under both normoxic and compared to hypoxic conditions, respectively.

Conclusions: We clearly demonstrate the existence of CTLA-4 on hMSC and its functionality with regard to the inhibition of PHA-induced TNF α secretion by PBMC. We also demonstrate that the expression of CTLA-4 (i) contributes to the immunomodulatory capacity of hMSC and (ii) supports the 'immune privileged' status of these cells.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4078

FRI0020 ALTERED BIOENERGETICS, MITOCHONDRIAL FUNCTION AND PRO-INFLAMMATORY PATHWAYS IN RA SYNOVIUM IN RESPONSE TO TOFACITINIB

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Background: Rheumatoid arthritis (RA) is a chronic joint disease, characterised by synovial inflammation and a shift in the metabolic profile of cells to a more destructive phenotype. The JAK-STAT signalling pathway is implicated in the pathogenesis of RA.

Objectives: To examine the effect of tofacitinib, a selective JAK inhibitor, on synovial cellular bioenergetics, mitochondrial function and subsequent pro-inflammatory mechanisms in RA.

Methods: Ex-vivo RA whole tissue synovial explants and primary RA synovial fibroblasts (RASFC) and were cultured with tofacitinib (1 μ M) for 24–72hrs. RASFC metabolism was assessed by the XF24-Flux-analyser and mitochondrial respiration was quantified using a mitochondrial random mutation capture assay. Mitochondrial function was assessed for reactive oxygen species (ROS), mitochondrial membrane potential (MMP) and mitochondrial mass (MM) using the specific cell fluorescent probes and differential gene expression by mitochondrial gene arrays or RT-PCR. Mitochondrial structural morphology was assessed by transmission electron microscopy. Lipid peroxidation (4HNE) was measured by specific ELISA. Dual staining of pSTAT3 and mitochondrial marker Cox-IV was demonstrated by confocal microscopy. The effect of tofacitinib (1 μ M) in RA synovial explant on markers of cellular bioenergetics and pro-inflammatory mediators, including cytokines and growth factors were quantified by ELISA, MSD multiplex assays and Real-time PCR.

Results: An initial screen demonstrated alterations in 18 key genes involved in mitochondrial function in RA synovial tissue in response to tofacitinib. Supporting this, tofacitinib inhibited ROS production, decreased the MMP and MM (all p<0.05), coupled with altered mitochondrial morphology. No effect observed for mtDNA mutations or 4HNE levels. Tofacitinib significantly inhibited the expression of glycolytic genes HIF1 α , HK2, LDHA, GSK3A and PDK1 (all p<0.05) suggesting altered energy metabolism. This was paralleled by inhibition of baseline ECAR (glycolysis) with a concomitant increase in baseline OCR (oxidative phosphorylation), ATP production, maximal respiratory capacity and in the respiratory reserve in RASFC, confirming a bioenergetic switch in synovial cells in response to tofacitinib. Furthermore, we demonstrated co-localisation of pSTAT3 with Cox-IV in RASFC, suggested that in addition to nuclear transcription, pSTAT3 may also act as a mitoTF, regulating mitochondrial function directly. Finally, in RA whole tissue explants, tofacitinib significantly inhibited glycolytic genes HK2, GSK3A and PDK1 which was paralleled by a significant decrease in the spontaneous secretion of inflammatory mediators IL-6, IL-8, IL-1 β , ICAM-1, VEGF, Tie2 and MMP1 (all p<0.05).

Conclusions: In this study, we describe a potential mechanism of action for tofacitinib, through reversing mitochondrial dysfunction and subsequent switch in cellular bioenergetics, in favour of a less glycolytic microenvironment leading to the reduction of inflammatory mediators. Thus, we have demonstrated that pathological cellular metabolism may be reversed by therapeutic treatment with tofacitinib.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6209

FRI0021 INTRAVENOUS INFUSION OF FUCOSYLATED BONE MARROW MESENCHYME CELLS IN PATIENTS WITH OSTEOPOROSIS. PRECLINICAL STUDY IN A MURINE MODEL

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Background: Osteoporosis is a skeletal disease characterized by systemic bone loss with an increased risk to bone fractures, associated with high morbidity and mortality¹. Mesenchymal stem cells (MSC) from bone marrow (BM) are ideal candidates for the treatment of osteoporosis because they are able to differentiate to osteoblasts although its osteotropism is low². Ex vivo fucosylation (adding a fucose residue) in α 1–3 position of the CD44 antigen by use of the enzyme fucosyltransferase VI (FTVI) yields Hematopoietic cell E and L-selectin ligand (HCELL) in MSC increasing the affinity for E-selectin and osteotropism.

Objectives: To describe the safety and efficacy of human fucosylated MSC infused in an immunocompromised mice model (NOD/SCID).

Methods: 31 NOD/SCID mice were randomized to by tail vein injection: 1x10⁶ fucosylated MSC (n=13), 1x10⁶ MSC (n=14) or saline (n=4). Toxicities were evaluated by a clinical score, weight and histological assessment (heart, lung, liver, spleen, kidney, gonads, brain, bone and BM). RT-PCR array-based evaluation of the expression of human β -actin and β 2-microglobulin genes was performed to study biodistribution of MSC. The maintenance of genetic integrity was evaluated during in vitro culture by karyotype. Additional samples of tibia and calota were to immunostained with a polyclonal Rabbit anti-human osteocalcina to demonstrate efficacy. Osteocalcin-positive cells were identified by a dark-brown cytoplasmic precipitate.

Results: There was no unexpected death and none of the mice had any acute toxicity. The histology of heart, liver, kidney, spleen, gonads, brain, bone and bone marrow was normal in all mice. There were localized areas of lung inflammation in 15%, 42% and 25% of mice infused with fucosylated MSC, MSC, and saline, respectively without significance differences (p=0.28). Biodistribution was normal with except one mice (infused with MSC non-fucosylated) showed an expression of human RNA β -actin and β 2-microglobulin in the lung sample taken after 12 weeks of intravenous infusion. Osteoblasts were seen in 100% of mice infused with fucosylated MSC, in 62.5% of animals infused with MSC alone, and none in saline group (p=0.01). NOD/SCID mice infused with fucosylated MSC presented a higher number of osteoblasts positive for osteocalcin in 10 high-power fields (400x) of tibia and calvarium sections that non-fucosylated (32 vs 5.5) (p=0.0082). Human osteoblasts were detected inside the bone from the fifth to the twelfth week after infusion.

Conclusions: Intravenous infusion of human fucosylated MSC is safe and effective in guiding the cells to bone with a higher potential to ossification in NOD/SCID mice that non-fucosylated MSC. These results are allowing to start a human clinical trial with intravenous fucosylated MSC as treatment in patients with osteoporosis.

References:

- [1] Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int* 2006 Dec;17(12):1726–33.
- [2] Connolly JF. Injectable bone marrow preparations to stimulate osteogenic repair. *Clin Orthop Relat Res* 1995 Apr;(313):8–18.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2945

FRIDAY, 16 JUNE 2017

Rheumatoid arthritis - etiology, pathogenesis and animal models

FRI0022 GLUTAMINE METABOLISM PLAYS A KEY ROLE IN THE CELL GROWTH OF FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

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Background: Many signaling pathways activated under inflammatory and hypoxic conditions have profound effects on intracellular metabolism to support cell growth and survival. The recent findings of cancer-specific metabolic changes, including increased glucose and glutamine consumption, has provided new therapeutic targets for consideration. The microenvironment in inflamed joints in RA is also characterized by hypoxia and low concentration of nutrients, and fibroblast-like synoviocytes from RA patients (RA-FLS) is known to have several tumor-like characteristics. However, the role of glucose and glutamine metabolism in the aberrant proliferation of these cells is unclear.