western blot analysis. Apoptosis was analyzing by annexin V (AV) and propidium iodide (PI) apoptosis detection kit by flow cytometry.

Results: As expected tofacitinib inhibited the expression of the phosphorylated form of STAT3 ($p < 0.01$). Rapamycin caused an increase in autophagy, while the levels of autophagy marker LC3-II were reduced after treatment with tofacitinib in vitro ($p < 0.001$ and $p = 0.02$, respectively, Figure 1B). In addition, the analysis of autophagic vacuoles by specific fluorescence dye confirmed the reduction of autophagy in RA-FLS treated with tofacitinib. The percentage of annexin V-positive apoptotic cells was not influenced by the treatment with tofacitinib.

Conclusion: The results of this study elucidated a new mechanism of action of tofacitinib related to autophagy modulation and led to a better understanding of the role of autophagy in RA. This study was supported by an unconditioned Research grant from Pfizer Inc.

REFERENCES

- [1] Burmester GR, Blanco R, Charles-Schoeman C, et al. Tofacitinib (CP-690,550) in combination with methotrexate in patients with active rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitors: a randomised phase 3 trial. *Lancet* 2013; 381: 451-60.
- [2] Vomerio M, Barbatì C, Colasanti T, et al. Autophagy and Rheumatoid Arthritis: Current Knowledge and Future Perspectives. *Front Immunol*. 2018;9:1577.
- [3] Shin YJ, Han SH, Kim DS, et al. Autophagy induction and CHOP under-expression promotes survival of fibroblasts from rheumatoid arthritis patients under endoplasmic reticulum stress. *Arthritis Res Ther*. 2010; 12: R19.
- [4] Maeshima K, Yamaoka K, Kubo S, et al. The JAK inhibitor tofacitinib regulates synovitis through inhibition of interferon- γ and interleukin-17 production by human CD4⁺ T cells. *Arthritis Rheum*. 2012;64:1790-8.

A. Image of fibroblast-like synoviocytes obtained from synovial tissue of RA patients during total knee replacement and used for in vitro experiments. B. Western blot analysis of autophagy marker LC3-II after in vitro treatment with tofacitinib.

Disclosure of Interests: Marta Vomerio: None declared, Mattia Caliste : None declared, cristiana barbatì: None declared, Tania Colasanti: None declared, francesca spinelli: None declared, Fulvia Ceccarelli: None declared, Carlo Perricone Speakers bureau: BMS; Lilly, Celgene, Sanofi, Annacarla Finucci: None declared, Mariangela Speziali: None declared, Alessandra Ida Celia: None declared, Michele Bombardieri Grant/research support from: Celgene, Consultant for: Medimmune, fabrizio conti: None declared, Guido Valesini: None declared, cristiano alessandri: None declared

DOI: 10.1136/annrheumdis-2019-eular.5445

AB0146

IDENTIFICATION A MARKED INFLAMMATION MEDIATED CARDIAC DYSFUNCTION DURING THE DEVELOPMENT OF ARTHRITIS IN COLLAGEN INDUCED ARTHRITIS MICE

Fang Wang, Aishu Luo, Zihao Zhou, Wenfeng Tan. *the First Affiliated Hospital of Nanjing Medical University, Nanjing, China*

Background: Systemic inflammation is strong linked to the increased risk of cardiovascular diseases (CVD) in rheumatoid arthritis (RA)¹. Increased cardiovascular risk in RA patients can not be fully explained by the traditional risk factors, such as hypertension, obesity, diabetes, age and smoking^{2,3}. Much more work is needed to elucidate the mechanism of chronic inflammation bridging RA and cardiovascular disease.

Objectives: To investigate the cardiac changes during the development of arthritis in collagen induced arthritis (CIA) mice and to explore the potential role of inflammation on cardiac dysfunction in RA.

Methods: Arthritis severity was evaluated using clinical indices, micro-computed tomography and histopathology. Cardiac function was determined by transthoracic echocardiography at week 5, 7, 9 and 11 after immunization in mice. At week 7 (day 50), mice joints and hearts were removed for pathological study, and cardiomyocytes and cardiac fibroblasts were isolated using Langendorff perfusion method *Ex vivo* to measure expression of inflammatory and cardiac-related genes by real time PCR. The expression of key molecule in cardiac dysfunction (β -MHC) was also tested in H9c2 cardiomyocytes treated with sera derived from CIA mice or RA patients.

Results: At day 50 after immunization, cardiac function in CIA mice was prominently reduced as evidenced by decreased ejection fraction (EF) and fractional shortening (FS), increased left ventricular end-systolic volume (LVESV) and internal systolic diameter (LVIDs). Accordingly, enhanced inflammatory cells infiltration and fibrosis were identified in ventricular tissues pathologically, and increased inflammatory genes expression including TNF- α , IL-6, IL-17 and MMP3 detected in isolated ventricular cardiomyocytes and cardiac fibroblasts from CIA mice. Furthermore, H9c2 cells treated with sera from CIA mice or RA patients exhibited high levels of β -MHC.

Conclusion: Joint inflammation is associated with an obvious cardiac dysfunction and enhanced inflammation infiltration and inflammatory cytokines production in cardiomyocytes and cardiac fibroblasts during CIA development. Our data provide the direct evidence that inflammation contributes to development of cardiac diseases in RA patients.

REFERENCES

- [1] SOLOMON DH, GOODSON NJ, KATZ JN, et al.: Patterns of cardiovascular risk in rheumatoid arthritis. *Ann Rheum Dis*. 2006;65:1608-12.
- [2] JAFRI K, BARTELS CM, SHIN D, GELFAND JM, OGDIE A: Incidence and management of cardiovascular risk factors in psoriatic arthritis and rheumatoid arthritis: A population-based study. *Arthritis Care Res (Hoboken)*. 2017;69:51-7.
- [3] AN J, ALEMAO E, REYNOLDS K, et al.: Cardiovascular outcomes associated with lowering low-density lipoprotein cholesterol in rheumatoid arthritis and matched nonrheumatoid arthritis. *J Rheumatol*. 2016;43:1989-96.

Acknowledgement: This project was sponsored by the grants from the National Natural Science Foundation of China (No. 81671610, 81471610, 81471611, 81801625)

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.4553

AB0147

RESEARCH PROGRESS OF MESENCHYMAL STEM CELL-DERIVED MICROVESICLES BY DELIVERING MICRORNA IN RHEUMATIC DISEASES

Na Zhang¹, Liyun Zhang², Gailian Zhang², Jin-Fang Gao³, MA Dan⁴, LI Juan⁵, Ke Xu¹. ¹Shanxi Dayi Hospital Affiliated to Shanxi Medical University, Taiyuan, China; ²Shanxi Dayi Hospital Affiliated to Shanxi Medical University, Taiyuan, China; ³Shanxi Dayi Hospital Affiliated to Shanxi Medical University, Taiyuan, China; ⁴Shanxi Dayi Hospital Affiliated to Shanxi Medical University, Taiyuan, China; ⁵Shanxi Dayi Hospital Affiliated to Shanxi Medical University, Taiyuan, China

Background: Recently, studies have shown that Mesenchymal Stem Cell-derived Microvesicles (MSC-MVs) can play a role in immune regulation and tissue repair by transporting miRNAs, and their effects are not weaker than that of their mother stem cells. Rheumatoid arthritis is an autoimmune disease characterized by chronic synovitis and progressive cartilage and bone destruction. Current treatments include non-steroidal anti-inflammatory drugs, glucocorticoids, DMARDs, and biological agents, but 30% of patients with rheumatism are ineffective, so there is an urgent need to find a new treatment.

Objectives: To review the research progress of MSC-MVs by delivering micro-RNA (miRNA) in rheumatic diseases.

Methods: Literatures were searched by using MSC-MVs, autoimmune diseases and miRNA as keywords.

Results: In the field of rheumatology, Chen C showed that after MSCT transplantation, mir-151-5p was transferred through exosomes to damaged bone marrow MSC of systemic sclerosis (SSc) mice, and the activation of IL4R/mTOR pathway was down-regulated to improve the osteoporosis of SSc mice^[1]. Exosomes of human synovial mesenchymal stem cells overexpressing miR-140-5p promoted cartilage regeneration and prevented OA in rat models^[2]. In the field of autoimmune diseases, Chen L et al. demonstrated that BMSC-derived exosomes containing miR-223 play a important role in liver protection by down-regulating NLRP3 (nod-like receptor family protein) and caspase-1 (caspase-1) in the experimental model of autoimmune hepatitis^[3]. Human BMSC-derived exosomes transfected with siFas and anti-mir-375 plasmids down-regulated Fas and miR-375 in human NOD scid gamma (NSG) mice, thereby protecting islet function and inhibiting immune rejection in islet transplantation^[4].

Conclusion: MiRNA targeting therapy with MSC-MVs as vector or the construction of MSC-MVs with overexpression of protective miRNA will provide new, accurate and promising treatment strategies for multiple rheumatic diseases.

REFERENCES

- [1] Chen, C., et al. Mesenchymal stem cell transplantation in tight-skin mice identifies miR-151-5p as a therapeutic target for systemic sclerosis. *Cell Res*, 2017. 27(4): p. 559-577. DOI:10.1038/cr.2017.11.

- [2] Tao S, C, Yuan T, Zhang Y. L, et al. Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model. *Theranostics*, 2017. 7(1): p. 180-195.
- [3] Chen L, et al. BMSCs-derived miR-223-containing exosomes contribute to liver protection in experimental autoimmune hepatitis. *Mol Immunol*, 2018. 93: p. 38-46. DOI:10.1016/j.molimm.2017.11.008.
- [4] Wen D, Peng Y, Liu D, et al. Mesenchymal stem cell and derived exosome as small RNA carrier and Immunomodulator to improve islet transplantation. *J Control Release*, 2016. 238: p. 166-175. DOI:10.1016/j.jconrel.2016.07.044

Acknowledgement: I confirm that there is no conflict of interest

Disclosure of Interests: Na Zhang: None declared, Liyun Zhang: None declared, Gaillian Zhang: None declared, Jin-Fang Gao Employee of: Shanxi Dayi Hospital Affiliated to Shanxi Medical University, Dan Ma: None declared, Juan Li: None declared, Ke Xu: None declared

DOI: 10.1136/annrheumdis-2019-eular.6452

AB0148 OSTEOBLASTS SECRETE A HIGH LEVEL OF OSTEOPROTEGERINE AND SUPPRESS OSTEOCLASTOGENESIS BY INHIBITING SOLUBLE RANKL – IMPLICATIONS FOR THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

Hiroaki Yazawa, Yoshimi Aizaki, Yasuto Araki, Kojiro Sato, Toshihide Mimura. Saitama Medical University, Department of Rheumatology and Applied Immunology, Saitama, Japan

Background: Osteoclasts (OCs) are multinucleated cells of monocyte/macrophage lineage and are the only cells known to resorb bone matrix. Osteoblasts (OBs) have the ability to differentiate bone marrow cells (BMCs) into osteoclasts when co-cultured with BMCs in the presence of Vitamin D₃ (1,25(OH)₂D₃) and prostaglandin E₂ (PGE₂). OBs produce both M-CSF, a survival factor for OC precursor cells, and RANKL, a differentiation factor. In rheumatoid arthritis (RA), synovial cells may fulfill the functional role of OBs, since OBs are not observed in the synovium of the joints. Although human BMCs are difficult to obtain, mouse BMCs are known to respond to human M-CSF and RANKL, and differentiate into OCs in the presence of both cytokines.

Objectives: Our aim was to examine whether a co-culture system of human synovial cells and mouse BMCs might be developed. Through this analysis, we attempted to clarify the characteristics of synovial cells in the pathogenesis of bone destruction observed in RA

Methods: Human synovial cells were prepared from synovium obtained in the course of joint replacement surgical operations performed at Saitama Medical University Hospital. Written informed consent, approved by the ethics committee of the hospital, was obtained from each patient prior to the experiment. Mouse OBs were obtained from the calvaria of newborn C57BL/6 mice. Mouse BMCs were co-cultured with either synovial cells or OBs. OCs were detected by tartrate-resistant acid phosphatase (TRAP) staining. The concentrations of M-CSF, RANKL and the decoy receptor for RANKL, OPG, were quantified by ELISA in the culture supernatant of synovial cells or OBs.

Results: When synovial cells and BMCs were cultured with 1,25(OH)₂D₃ and PGE₂, OCs were not induced. OC precursor cells did not seem to even survive. Next, we added human M-CSF to the system. This time, OC precursor cells remained, but TRAP-positive multinuclear cells were not observed. It was only in the presence of exogenous M-CSF and RANKL that BMCs transformed into OCs. RANKL was not detected in the culture supernatant of synovial cell culture, but OPG and M-CSF were detectable at a level comparable to mouse OBs. Finally, we cultured mouse OBs and BMCs that were separated by a polycarbonate membrane in the presence of 1,25(OH)₂D₃ and PGE₂. In this case, too, BMCs did not survive unless exogenous M-CSF was added, and they did not differentiate into OCs unless both M-CSF and RANKL were added to the system.

Conclusion: These results indicate that the M-CSF derived from OBs or synovial cells is not sufficient to support the survival of OC precursors. Likewise, the soluble RANKL derived from OBs is by itself insufficient for the differentiation of OCs, confirming the importance of a cell-cell interaction between OBs and OC precursors. Thus, membrane-bound RANKL seems to play a role more important than soluble RANKL in the co-culture system of OCs *in vitro*. Soluble RANKL may be neutralized by the OPG that is produced at a fairly high level from both OBs and synovial cells. We propose that this is the system by which ectopic differentiation of OCs is prevented.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.6410

AB0149 ARSENIC TRIOXIDE IMPROVES TREG AND TH17 BALANCE VIA MODULATING STAT3 IN TREATMENT-NAÏVE RA PATIENTS

Yue Zhang^{1,2}, Li Chunling¹, Zhiyi Zhang¹, on behalf of Precision medicine center for Rheumatic diseases, First clinical college of Harbin Medical University. ¹First Clinical College of Harbin Medical University, Rheumatology and Immunology, Harbin, China; ²Shenzhen Futian Hospital for Rheumatic Diseases, Shenzhen, China

Background: We have a long-term interest in novel arsenic trioxide As₂O₃ (ATO) medical application since some acute promyelocytic leukemia (APL) patients have been cured by first using ATO and survived for more than 45 years successfully treated by Prof. Zhang Tingting in our institute. Moreover, our previous studies have shown that ATO significantly suppress angiogenesis and induced fibroblast like synovial cells (FLS) apoptosis in collagen induced arthritis (CIA) model [1, 2]. However, the exact mechanism by which ATO anti-rheumatic and whether this occurs via modulation of immune system are still unclear.

Objectives: To investigate the immunologic mechanism by which ATO may inhibit T helper 17 cells (Th17) differentiation while promote regulatory T cells (Treg) generation via modulating signal transducer and activator transcription 3 (STAT3) in treatment-naïve RA patients.

Methods: Naïve CD4⁺T cells sorted by fluorescence-activated cell sorting (FACS) from treatment-naïve RA patients and healthy controls were used to investigate the effect of ATO on its polarization process and related cytokines. Knockdown or enforced expression of STAT3 transfection experiments were conducted by small interfering RNA (siRNA) and lentivirus STAT3 to verify the mechanism of ATO on Th17/Treg balance *in vitro*. Collagen-induced arthritis (CIA) model was constructed to detect the clinical score, histopathological change, bone destruction, Th17/Treg proportion and joint tissues immunohistochemistry. Single cell sequencing and other methods have been applied too.

Results: We found that ATO prevented activated naïve CD4⁺T-cell differentiate into Th17 cell and reduced cytokine production by activated Th17 cells by downregulating their signature transcription factors, STAT3 and orphan nuclear receptor (RORγt). Notably, ATO reduced Th17 cells frequency while increased Treg cells frequency under specific polarizing conditions from treatment-naïve RA patients by transfecting siRNA STAT3 and lentivirus STAT3. Furthermore, we have noticed that intervention of ATO in CIA model attenuated joint inflammatory infiltration and bone destruction, significantly improved the imbalanced Treg/Th17 ratio. In-detail single cell sequencing analysis is ongoing.

Conclusion: ATO may be a immune modulator candidate for treatment-naïve RA patients via balancing well Treg/Th17 cell ratio through STAT3 regulation.

REFERENCES

- [1] Zhang, J., et al., *Inhibition of angiogenesis by arsenic trioxide via TSP-1-TGF-beta1-CTGF-VEGF functional module in rheumatoid arthritis*. *Oncotarget*, 2017. 8(43): p. 73529-73546.
- [2] Mei, Y., et al., *Arsenic trioxide induces apoptosis of fibroblast-like synovial cells and represents antiarthritis effect in experimental model of rheumatoid arthritis*. *J Rheumatol*, 2011. 38(1): p. 36-43.
- [3] Vasheghani, F., et al., *PPARgamma deficiency results in severe, accelerated osteoarthritis associated with aberrant mTOR signalling in the articular cartilage*. *Ann Rheum Dis*, 2015. 74(3): p. 569-78.

Acknowledgement: National Science Foundation of China (NO. 81273291) and (NO. 81771748, 81771749)

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.8330

AB0116 BROMODOMAIN INHIBITOR, I-BET762 INHIBITS PRODUCTION OF PRO-INFLAMMATORY MEDIATORS BY DOWN-REGULATING BROMODOMAIN AS AN EPIGENETIC READER IN RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIAL CELLS

Ra Ham Kim¹, Hyun Jung Yoo^{1,2}, Shin Eui Kang¹, Ji Soo Park¹, Seon Uk Kim¹, Eun Young Lee², Jin Kyun Park², Yeong Wook Song^{1,2}. ¹Seoul National University, Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, and College of Medicine, Seoul, Korea, Rep. of (South Korea); ²Seoul National University Hospital, Division of Rheumatology, Department of Internal Medicine, Seoul, Korea, Rep. of (South Korea)

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disorder, characterized by joint inflammation and bone destruction. The fibroblast-like synovial cell contributes to the pathogenesis of RA through proliferation and production of cytokines. Recently, blockade of the bromodomain and extra-terminal domain