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

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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.

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

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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.

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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

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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