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-positi

tive 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 unconditional Research grant from Pfizer Inc.

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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 prostaglandinE₂(PGE₂). OBs produce both M-CSF, a survival factor for OC precursor cells, and RANKL, a differentiation factor. In rheumatoid arthritis (RA), synoviocytes 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 synoviocytes and mouse BMCs could function properly when treated with RA-related cytokines. We attempted to clarify the characteristics of synoviocytes in RA.

Methods: Human synoviocytes 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 synoviocytes or OBs. OBs 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 synoviocytes or OBs.

Results: When synoviocytes 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 synoviocyte culture, but OPG and M-CSF were detectable at a level comparable to mouse OBs. Finally, we co-cultured mouse OBs and BMCs that were separated by a poly-carbonate 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 synoviocytes 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 synoviocytes. We propose that this is the system by which ectopic differentiation of OCs is prevented.