**AB0122**

**RP53 INDUCES ECOTDOMAIN SHEDDING OF TNF RECEPTOR 1 AND THEREBY INHIBITS INFLAMMATORY RESPONSES IN RHEUMATOID FIBROBLAST-LIKE SYNOVIOCYTES**

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**Background:** Rheumatoid arthritis (RA) is a chronic inflammatory disease by autoimmunity disorder that primarily affects joints. Usually RA has been treated with disease modifying anti-rheumatic drugs but biological response modifiers has developed the treatment of RA. Among these anti-tumour necrosis factor (TNF) agents were the first to be successfully used in treating RA. Various anti-TNF-α therapy might be lead to substantial functional improvement in RA patients.

**Objectives:** We studied the isolated two polypeptides (Rp53, Rp54) from Rubia philippinensis, traditional medicine plant, about anti-inflammation effects in fibroblast-like synoviocytes (FLS) derived from patients with RA.

**Methods:** The effects of polypeptides on anti-inflammation were measured by cytokine assay kits (TNF-α, IL-6). The underlying for NF-κB signalling pathway was examined by western blot and NF-κB reporter activity. We examined the effect of Rp53 on the formation of the TNFR1 signalling complex, recruitment of TRADD, RIP in response to TNF by immunoprecipitation experiments. To determine whether Rp53 induced TNF receptor 1 shedding were exposed to these compounds for 1 hour, and then culture media and cell lysates were analysed by Western blotting using anti-TNF receptor 1 antibody.

**Results:** Pretreatment with Rp53 resulted in a remarkable decrease of the secretion of TNF-induced proinflammatory cytokines TNF-α and IL-6 in RA-FLS. Rp53 strongly inhibited the nuclear factor κB (NF-κb) signalling pathway induced by TNF-α, but not that induced by IL-1β. Analysis of the upstream signalling events affected by Rp53 revealed that it strikingly inhibited the TNF-induced recruitment of TNFR1-associated death domain protein (TRADD) and receptor-interacting protein (RIP) to TNFR1. Rp53 reduced the interaction with TNFR1 to TNF cytoskeleton and enhanced the activation of the p38 mitogen-activated protein (MAP) kinase. Rp53, the polypeptides induced the proteolytic cleavage of TNF-R1 and its receptor-interacting protein (RIP) to TNFR1. Rp53 reduced the interaction with TNFR1 to TNF cytoskeleton and enhanced the activation of the p38 mitogen-activated protein (MAP) kinase.

**Conclusions:** This study demonstrates that the TACE-dependent ectodomain shedding of TNF receptor 1 through the activation of p38 MAP kinase, and thereby inhibits the TNF-α induced NF-κB signalling pathway in RA-FLS cells.

**Disclosure of Interest:** None declared

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**AB0124**

**DETECTION OF PRECURSORS OF RANK-osteoclast-like cells (OLCs) IN PERIPHERAL BLOOD AND OLCs IN BONE TISSUE FROM RHEUMATOID ARTHRITIS PATIENTS**

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**Background:** Previously, we reported that novel osteoclast-like cells (OLCs) were induced, both in vitro and in vivo, from mouse bone marrow-derived macrophages (BMMs) by addition of a combination of TNFα and IL-6. Recently, O’Brien et al showed that TNF/IL-6 can drive osteoclastogenesis in BMMs from RANK-deficient mice.

**Objectives:** We aimed to examine the differentiation of OLCs, which were induced by a combination of TNFα and IL-6 from human peripheral blood mononuclear cells (PBMCs) and CD14 +monocytes to identify differences in molecular expression patterns between OLCs and conventional osteoclasts. Furthermore, we identified OLCs and osteoclasts on the bone tissue of the joint in patients with rheumatoid arthritis (RA).

**Methods:** PBMCs and CD14 +monocytes from healthy volunteers and/or RA patients were stimulated with TNFα and IL-6 or RANKL. Quantitative RT-PCR was used to measure mRNA expression levels of RANK, cathepsin K, calcitonin receptor, and dendritic cell-specific transmembrane protein. Prepared undecalci-fied tibial bone from 6 RA patients and 6 OA patients undergoing joint surgery were stained by tartrate-resistant acid phosphatase (TRAP) staining and immunohistochemistry with anti-RANK antibody, expression of which were analysed. Osteoclasts and OLCs were identified as multinucleated TRAP+/RANK ±cells and TRAP+/RANK- cells, respectively, adherent to the bone surface.

**Results:** The number of OLCs treated with a combination of TNFα and IL-6 from PBMCs or CD14 +monocytes in RA patients was significantly increased compared to that in healthy volunteers. Expression levels of RANK mRNA were clearly up-regulated in osteoclasts, and was obviously down-regulated in OLCs compared to that in osteoclast precursors. In cancellous bone, the number of TRAP-/RANK- osteoclasts was significantly increased in RA patients compared to that in OA patients. Interestingly, numerous TRAP-/RANK- OLCs were present in the cancellous bone of RA patients, while almost none were observed in the cancellous bone of OA patients.

**Conclusions:** The combination of TNFα and IL-6 strongly induced the differentiation of OLCs from PBMCs or CD14 +monocytes in RA patients. OLCs was charac-terised with TRAP+/RANK- multinucleated cells, which can distinguish conventional TRAP+/RANK+ multinucleated osteoclasts. TRAP+/RANK- OLCs also were present in the bone tissue of RA patients. These results suggest that conventional osteoclasts and novel OLCs could be involved in the pathogenic mechanisms of inflammatory bone destruction such as RA.

**References:**