REFERENCE:

- [1] Clarke DL, et al. *Fibrogenesis & Tissue Repair* 2013, 6:20. 2. Schafer PH, et al. *Cell Signal.* 2016;28:753-63. 3. Carthy JM. *J Cell Physiol.* 2018;233:98-106.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.7505

THU0054

THE SYNOVIAL FLUID FROM RHEUMATOID ARTHRITIS PATIENTS CONTAINS SOLUBLE TAM RECEPTOR TYROSINE KINASES OF WHICH SOLUBLE TYRO3 AND ITS LIGAND GAS6 CORRELATES WITH THE PROINFLAMMATORY CYTOKINE LEVELS

Julia Vullings, Claire Waterborg, Marije Koenders, Peter van Lent, Peter van der Kraan, Fons van de Loo. *Radboud Institute for Molecular Life Sciences (RIMLS), Rheumatology, Nijmegen, Netherlands*

Background: We recently showed that the tyrosine-protein kinase receptors MER and AXL, both members of the TAM (including TYRO3) receptor family, play a protective role in mouse models of rheumatoid arthritis (RA) [1, 2]. In both humans and mice, TAM receptors can be proteolytically cleaved from the cell membrane and shed as a soluble (s) receptors. These sTAM receptors may act as decoy receptors, bind their ligands Growth Arrest-Specific 6 (GAS6) and Protein S (PROS1), and thereby inhibit the immunoregulatory and anti-inflammatory effect of TAM receptor activation, and prevent TAM receptor-mediated phagocytic clearance of dying cells.

Objectives: To determine the soluble TAM receptor levels in synovial fluids from RA and osteoarthritis (OA) patients and correlate this to the inflammatory process.

Methods: The level of sMER, sAXL, sTYRO3, GAS6, IL-8, TNF α and IL-1 β was measured in synovial fluids of RA (n = 28) and OA patients (n = 12) by ELISA or multiplex array. Synovial explants were cultured for 24 hrs and the release of sMER and GAS6 was measured by ELISA. Human synovial explants of RA (n = 15) and osteoarthritis (OA) (n = 17) patients were immunostained for MER.

Results: Soluble MER and sTYRO3 were significantly enhanced in synovial fluids (P<0.0001 and P=0.0042) of RA patients as compared to OA patients. The sAXL and GAS6 levels in synovial fluid of RA and OA patients was equally high (P = 0.267 and P = 0.074). In RA patients, but not in OA patients, the synovial fluid levels of sTYRO3 and GAS6 (a ligand for all three TAM receptors) positively correlated with TNF α (r=0.555, r=0.477), IL-1 β (r=0.597, r=0.481) and IL-8 (r=0.567, r=0.529). Soluble MER levels, however, did not correlated with local inflammatory markers. Although synovial explants from RA released more sMER into the culture media than explants from OA (P = 0.0045), the explants of both RA and OA patients contained comparable amounts of immunopositive MER cells.

Conclusion: We showed that synovial fluid sTYRO3 and GAS6 levels correlates with higher proinflammatory cytokine levels in RA but not in OA patients. This is in line with the recent observation done by Xu et al. [3], that circulating levels of sTYRO3, but not sMER or sAXL, correlates with disease activity and bone destruction in RA patients. In accordance to serum, the synovial sMER levels were significantly higher in RA - than in OA patients with sAXL levels comparable between both patient groups. Interestingly, only for sMER the synovial fluid levels were much higher than in serum suggesting local production as we confirmed in the synovial explants cultures. This illustrates that release of soluble TAM receptors is either the results of different processes (enzymatic shedding or alternative splicing isoforms) or reflects the different cell populations in the arthritic joint. We found that naïve macrophages express high MER and inducible AXL, while synovial fibroblast express high AXL and TYRO3. Further studies are warranted to determine if sMER levels impair the clearance of apoptotic cells in the arthritic joint as we have found in our mice studies. Our study shows that synovial sTYRO3 is a marker for joint inflammation and possibly a novel therapeutic target for RA.

REFERENCES:

- [1] Waterborg, C.E., et al., *Protective role of the Mer Tyrosine Kinase via efferocytosis in rheumatoid arthritis Models.* *Frontiers in immunology*, 2018. 9.

- [2] Waterborg, C.E., et al., *03.05 The tam receptors axl and mer play a protective role in a temporal and spatial manner in inflammatory arthritis.* 2017, BMJ Publishing Group Ltd.
- [3] Xu, L., et al., *Soluble TAM receptor tyrosine kinases in rheumatoid arthritis: correlation with disease activity and bone destruction.* *Clinical & Experimental Immunology*, 2018. 192(1): p. 95-103.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.7288

THU0055

TPL2 INHIBITION SUPPRESSES MEK-ERK INFLAMMATORY SIGNALING AND PROINFLAMMATORY CYTOKINE PRODUCTION IN PRIMARY HUMAN MONOCYTES

Matthew R. Warr, Angie Hammond, Grace Park, Nathan Wright, James Taylor. *Gilead Sciences, Inc., Foster City, United States of America*

Background: Tumor progression locus 2 (TPL2, also known as MAP3K8) is a mitogen-activated protein kinase kinase kinase and the primary regulator of ERK-mediated gene transcription downstream of multiple proinflammatory stimuli including bacterial products (eg, LPS and bacterial peptidoglycans), damage-associated molecular patterns (DAMPs), TNF α , and IL-1 β .¹ Dysregulated signaling downstream of these inflammatory signals can drive uncontrolled immune cell activation and inflammation, which is associated with multiple chronic inflammatory and autoimmune diseases. As such, TPL2 inhibition represents a strategy to modulate inflammation in a variety of disease settings.

Objectives: We evaluated the effect of a highly selective TPL2 inhibitor on inflammatory signaling and cytokine production in LPS and TNF α -stimulated primary human monocytes.

Methods: Monocytes were precultured with a TPL2 inhibitor and stimulated with LPS or TNF α , and phosphosignaling and cytokine production were then evaluated at 30 minutes and 4 hours poststimulation. A431 cells (human epidermoid carcinoma cell line) were stimulated with TNF α or EGF and phospho-ERK was evaluated after 30 minutes.

Results: TPL2 inhibition selectively inhibited LPS and TNF α -stimulated phosphorylation of TPL2, MEK, and ERK, with little to no inhibition of phosphorylated p38, JNK, or p65 observed. TPL2 inhibition similarly inhibited both the RNA production and secretion of TNF α , IL-1 β , IL-6, and IL-8 following LPS stimulation in primary human monocytes. To confirm TPL2 requirement for inflammatory, but not Ras-mediated (growth factor stimulated) ERK signaling, A431 cells were stimulated with either TNF α or EGF. Although TPL2 inhibition reduced TNF α -stimulated pERK, no effect on ERK activation downstream of EGF was observed.

Conclusion: This work demonstrates the selective effects of TPL2 inhibition on ERK-mediated signaling and proinflammatory cytokine gene transcription in primary human monocytes and highlights the potential for TPL2 inhibition to treat diseases associated with dysregulated inflammatory signaling and chronic inflammation.

REFERENCE:

- [1] Gantke T, Sriskantharajah S, Ley S. *Cell Res.* 2011;21:131-145.

Disclosure of Interests: Matthew R. Warr Shareholder of: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc., Angie Hammond Grant/research support from: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc., Grace Park Grant/research support from: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc., Nathan Wright Shareholder of: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc., James Taylor Grant/research support from: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc.

DOI: 10.1136/annrheumdis-2019-eular.2512