

A39 TOLL-LIKE RECEPTOR-4 SIGNALLING IS SPECIFICALLY TAK1-INDEPENDENT IN SYNOVIAL FIBROBLASTS

Ben T van den Brand,¹ Shahla Abdollahi-Roodsaz,¹ Onno J Arntz,¹ Michael Kracht,² Jeroen Geurts,¹ Wim B van den Berg,¹ Fons A J van de Loo¹ ¹*Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands;* ²*University Giessen, Rudolf-Buchheim-Institute of Pharmacology, Frankfurter Strasse 107, 35392 Giessen, Germany*

10.1136/ard.2010.148965.9

Background and objective Activated synovial fibroblasts are key players in the pathogenesis of rheumatoid arthritis (RA) by driving inflammation and joint destruction. Numerous molecules including cytokines and toll-like receptor (TLR) ligands induce pro-inflammatory signalling and gene expression through a hierarchical network of kinases. Upstream mitogen-activated protein kinase kinase kinases (MAP3Ks) represent an attractive target for RA treatment. In this study the authors sought to determine the role of the MAP3K transforming growth factor β activated kinase1 (TAK1) in cytokine and TLR-mediated signalling.

Materials and methods TAK1 activity was inhibited using either a small molecule inhibitor 5Z-7-oxozeaenol or lentivirally overexpressed kinase-inactive TAK1-K63W mutant in murine embryonic and human dermal and synovial fibroblasts. Fibroblasts were stimulated with interleukin 1 (IL-1), tumour necrosis factor (TNF), TLR2 or TLR4 agonists and responses were evaluated using transcriptional reporters and analysis of gene expression of collagenases (matrix metalloproteinases (MMP3, MMP13)), cytokines (IL-1 β , IL-6) and chemokines (IL-8, MCP-1).

Results TAK1 inhibition abrogated cytokine- and TLR-induced activation of nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) and *Saa3*-promoter reporters in mouse and human

dermal fibroblasts. In synovial fibroblasts, TAK1 crucially regulated IL-1 and TNF mediated NF- κ B, but not *Saa3*-promoter activation. Furthermore, TAK1 was required for inducible mRNA expression of IL-1 β , IL-6, IL-8, MMP3 and MMP13, but not MCP-1, in response to IL-1, TNF and TLR2 agonist. Unexpectedly, TLR4-induced NF- κ B activation and gene expression was fully TAK1-independent as found using the TAK1 inhibitor or kinase inactive mutant. Western blots demonstrated IL-1-inducible p38 and p65 phosphorylation and inhibition by the TAK1 inhibitor 5Z-7-oxozeaenol, whereas no effect of TAK1 inhibitor on lipopolysaccharides signalling in RA-SF.

Conclusions In general, TAK1 plays a prominent role in regulation of IL-1- and TNF mediated signalling in fibroblasts. Interestingly, TLR4 signalling is specifically TAK1-independent in synovial fibroblasts. Consequently, therapeutic TAK1 inhibition in arthropaties may not dampen the damage-associated molecular pattern-mediated TLR4 activation of synovial fibroblasts.