**A96 METASTASIS-PROMOTING PROTEIN S100A4 (MTS1)
UPREGULATES PRODUCTION OF PROINFLAMMATORY
CYTOKINES VIA TLR-4**

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Background The authors have previously demonstrated that metastasis inducing protein S100A4 is upregulated in plasma, synovial fluid as well as in synovial tissue from patients with rheumatoid arthritis (RA). This protein contributes to the pathogenesis of RA by inducing expression of matrix degrading enzymes and regulating apoptosis of synovial fibroblasts.

Aim The authors hypothesised that S100A4 might also regulate function of other cells and therefore evaluated the effect of S100A4 on peripheral blood monocytes.

Methods CD14 monocytes were selected from human peripheral blood mononuclear cells from patients with RA. Monocytes were stimulated with S100A4 dimer (1 µg/ml) and multimer (0.5–2 µg/ml). Gene expression of proinflammatory cytokines (tumour necrosis factor α (TNF α), interleukin (IL)1 and IL6) were assessed using real-time PCR. The protein levels in culture supernatants were determined by ELISA. To demonstrate binding of S100A4 to either advanced glycation end products receptor (RAGE) or Toll-like receptor (TLR)-4, anti-human RAGE and TLR-4 antibodies were added to adherent monocytes prior to treatment with the S100A4 multimer.

Results Stimulation of monocytes with S100A4 multimer significantly upregulated TNF α (11-fold), IL1 (6.5-fold) and IL6 (75-fold) mRNAs compared with unstimulated monocytes and cells stimulated with S100A4 dimer after 6 h ($p < 0.01$). Most interestingly, S100A4 multimer induced significant release of TNF α to the cell culture medium compared with dimer (4474.6 ± 1282 vs 958.4 ± 706.2 pg/ml, $p < 0.01$) and control (4474.6 ± 1282 vs 250.3 ± 165 pg/ml, $p < 0.001$). Anti-RAGE antibodies did not change, however TLR-4 blockade significantly decreased production of TNF α by monocytes treated with S100A4 multimer.

Conclusion Our findings demonstrate S100A4 as a potent immune mediator stimulating production of key proinflammatory cytokines that are major players in the pathogenesis of RA.