ELISAs were performed in triplicate for IL-6 TIMP-1, and MMP-1 levels. In some experiments cells were pretreated with 100 μM of WRW4, a specific formyl peptide receptor inhibitor with A-SAA. Also we used fibroblasts derived from a patient with a genetic non sense mutation that results in no IRAK-4 protein production and hence a halt in TLR signalling. IRAK-4 is a central downstream mediator of Toll-Like Receptor mediated signalling and is crucial for such signalling.

Results Healthy human dermal fibroblasts incubated with A-SAA secreted high levels of IL-6 compared to untreated control cultures. Moreover A-SAA induced increased levels of TIMP-1 both at the mRNA levels and also the protein levels as determined by ELISA. Levels of the target of TIMP-1, MMP-1 protein levels were not altered at all. Thus the effect leads to a shift in the ratio of TIMP-1 to MMP-1 favouring ECM deposition. Pretreatment with WRW4 prior to A-SAA did not alter IL-6 or TIMP-1 expression levels, showing that the formyl peptide receptor plays no role in TIMP-1 induction mediated by addition of A-SAA. Furthermore cells derived from a gene deleted IRAK-4 patient with no IRAK-4 protein stimulated with A-SAA compared to control fibroblasts had TIMP-1, IL-6 levels compared to non-treated (media alone) dermal fibroblasts.

Conclusions A-SSA induces TIMP-1, but importantly does not alter levels of TIMP-1 target MMP-1, thus shifting the TIMP-1/ MMP-1 ratio. IL-6, a classic proinflammatory cytokine involved in SSc pathogenesis, is also elevated by A-SAA treatment in vitro. The signalling involved IRAK-4, a critical downstream messenger of TLR mediated signalling, but not formyl peptide receptors.