

TITLE: TNFAIP6 and SAA1 mRNA levels are elevated in peripheral blood cells following destabilising knee injuries.

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Purpose: Acute destabilising injury to the joint is a considerable risk for the development of osteoarthritis (OA); about 50% of individuals with such injuries will develop OA within 5 to 10 years. It is currently impossible to predict whether an individual with an acute destabilising joint injury will develop OA or not. Our previous work in mice has revealed a number of highly mechanosensitive genes/pathways that are regulated early upon surgical destabilization of the medial meniscus (DMM). As many of the genes identified were ubiquitously expressed, inflammatory response genes, we asked whether they were also regulated in the cellular compartment of the peripheral blood. Finally, we examined whether genes that were regulated in the mouse blood upon DMM were also regulated in the peripheral blood of patients who had recently sustained a destabilising knee injury.

Methods: Blood was taken and RNA extracted from male 10 week old C57BL6/J mice 1, 3 and 6 days following either sham, or DMM surgery. Gene expression changes were examined for 47 genes, previously shown to be regulated in the destabilised joint, at each time point using hydrolysis-probe low density arrays. RNA was also isolated from 30 individuals who had sustained recent knee injury and 30 healthy, age- and sex- matched controls. The latter were selected from participants of the Knee Injury Cohort at the Kennedy (KICK) study. All those aged 16-50, with significant knee injury excluding fracture within the last 20 days, no evidence of OA in the knee or other joints, no Non-Steroidal Anti-Inflammatory drug use within the last 7 days, no current infections, other injuries or surgery in the last 3 months were selected for this sub-study. Protein levels of TNF alpha induced protein 6 (TNFAIP6, also known as TSG6) and serum amyloid A-1 (SAA-1) in serum and synovial fluid were quantified by electrochemiluminescence (MesoScale Discovery, USA).

Results: CD14, arginase 1 (ARG1), serum amyloid A-3 (SAA3) and TNFAIP6 were regulated in the cellular compartment of the peripheral blood of mice. All four genes were initially down-regulated at 3 days post-surgery, but at 6 days post-surgery, their levels were elevated in the blood of mice that had undergone DMM compared with sham surgery. Examination of human blood samples revealed that, of the 4 genes regulated in the mouse, only SAA1 (the human homologue of SAA3) (1.78 fold, 1.04-3.04, $p=0.027$, 2-way ANOVA with Sidak post hoc) and TNFAIP6 (1.81 fold, 1.05-3.09, $p=0.0201$, 2-way ANOVA with Sidak post hoc) were regulated early upon acute knee injury compared with controls. Quantification of protein levels for SAA1 and TNFAIP6/TSG-6 in the serum and synovial fluid from the same individuals revealed an inverse correlation between the transcripts of SAA1 in the blood and the levels of TNFAIP6 in the intra-articular compartment of the same patients (Spearman $r=-0.4674$, $p=0.0161$).

Conclusions: We have identified two genes regulated in the blood early after severe knee injury in both mice and humans. At least in mice, the expression of these genes appears to be specifically associated with joint destabilisation rather than surgery per se, as they were not regulated in blood from sham-operated mice. As these genes were originally identified from injured joint extracts, it is tempting to speculate that peripheral blood cells may provide an easily accessible tissue from which joint responses can be examined. As TNFAIP6 has already been implicated as a biomarker of disease severity in established OA, further work is ongoing to establish whether these molecules are predictive of OA risk after acute knee injury.