Alarmins S100A8 and S100A9 elicit a catabolic effect in human osteoarthritic chondrocytes that is dependent on TLR4

INTRODUCTION:
S100A8 and S100A9 are two Ca\(^{2+}\)-binding proteins classified as damage associated molecular patterns (DAMPs) or alarmins that are found in high amounts in the synovial fluid of osteoarthritic (OA) patients. Previously, we found that S100A8 and S100A9 are associated with cartilage degradation in murine collagenase-induced OA, a model in which synovial activation is an important hallmark. We also showed that S100A8 and S100A9 stimulate expression and activity of matrix metalloproteinases (MMPs) and pro-inflammatory cytokines in murine chondrocytes. In the current study, we investigated whether and via which receptor S100A8 and/or S100A9 can have a catabolic effect on chondrocytes from OA patients.

METHODS:
Using immunohistochemistry, we stained for S100A8 and S100A9 protein, MMPs and a cartilage breakdown epitope specific for MMPs (VDIPEN) in cartilage from OA donors. Isolated chondrocytes or explants from OA and non-OA donors were stimulated with S100A8 and/or S100A9. mRNA and protein levels of MMPs, cytokines and cartilage matrix molecules were determined with RT-qPCR and Luminex. For receptor blocking studies, specific inhibitors for Toll-like receptor 4 (TLR4)- (intracellular TAK242) and RAGE and carboxylated glycans (blocking antibodies) were used. The Medical Ethics Committee of Radboud University Nijmegen Medical Centre approved the study protocol. Statistical differences were calculated with either paired t-test or Mann Whitney U test, as indicated, using Graph Pad Prism 5.

RESULTS:
In cartilage of OA patients, localisation of S100A8 and S100A9 protein was found close to chondrocytes and was associated with proteoglycan (PG) depletion as measured by safranin O staining. MMP1 and -3 and VDIPEN expression. Stimulation of chondrocytes with S100A8 and S100A9 caused a significant upregulation at the mRNA level of MMP1, -3, -9 and -13 (5.7, 5.0, 4.0, and 3.1-fold respectively) (Figure 1) and downregulation of anabolic markers aggrecan and collagen type II (2.7 and 2.7-fold decrease respectively).

The upregulation of MMPs was confirmed on the protein level (2.3, 3.1 and 3.6-fold respectively). Moreover, S100A8 and S100A9 caused a huge increase in cytokine and chemokine expression. IL-6, IL-8 and MCP-1 were all greatly increased by S100A8 and S100A9 at both the mRNA (15.1, 24.0 and 3.7-fold respectively) as well as the protein level (29.1, 49.7 and 7.7-fold respectively) (Figure 2). Together, upregulation of MMPs and cytokines and downregulation of production of cartilage matrix molecules favor cartilage loss by the OA chondrocyte. Blocking TLR4 inhibited the upregulation of MMPs, IL-6, IL-8, MCP-1 and collagen type II by S100A9 in OA chondrocytes (Figure 3). In contrast, the blocking of carboxylated glycans and RAGE did not alter the S100-effects.

Finally, the catabolic effect of S100A8 and S100A9 was significantly more pronounced in chondrocytes from OA patients when compared to non-OA as measured by MMP3 (Figure 4) and aggrecan mRNA. TLR4 mRNA expression was enhanced in OA chondrocytes, which might explain the increased sensitivity.

DISCUSSION:
S100A8 and S100A9 have a catabolic effect on human chondrocytes that is dependent on TLR4. OA chondrocytes are more sensitive for non-OA as measured by MMP3 (Figure 4) and aggrecan mRNA. TLR4 mRNA expression was enhanced in OA chondrocytes, which might explain the increased sensitivity.

SIGNIFICANCE:
During OA the erosion of cartilage is an important cause of pain and disability in the joint. Elucidation of the role of S100A8 and S100A9 in cartilage damage could lead to new treatments and relieve the burden for the growing population of people who suffer of this debilitating disease.