Alarmins S100A8/A9 Regulate Osteophyte Formation At Intermediate And Late Stages Of Experimental Osteoarthritis With High Synovial Activation

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Disclosures:

Introduction: The main pathological feature of osteoarthritis (OA) is degradation of the articular cartilage. However, other important hallmarks include subclinical inflammation of the synovium, sclerosis of subchondral bone and ectopic formation of new bone and cartilage at the ligaments or joint margins, termed enthesophytes and osteophytes, respectively. Enthesophytes/osteophytes limit joint movement and cause pain. Biologically, they reminisce endochondral ossification with similar phases: chondrogenesis, chondrocyte hypertrophy and ossification among others (1). S100A8 and S100A9 are Ca2+-binding proteins that can be released by monocytes and (synovial) macrophages in large quantities thereby alarming the immune system. Hence, S100A8/A9 are classified as “alarmins” or damage associated molecular patterns (DAMPs). In previous studies at our institute, we found that S100A8 and A9 are involved in synovial activation and cartilage degradation during human and murine experimental OA.

In the current study, we explored the active involvement of S100A8/A9 in osteophyte formation in experimental OA models that differ in degree of synovial activation.

Methods: Experimental OA was elicited in C57Bl/6 (wild-type) mice and S100A9/-/- mice (in which peripheral myeloid cells also lack functional S100A8), either by two times intra-articular injection of 5 µg collagenase (CIOA), or by transection of the anterior meniscotibial ligament (DMM). Osteophyte size was assessed by a blinded observer on Safranin O stained sections using Leica Application Suite (LAS) image analysis software. Chondrogenesis was induced by bringing murine C3H10T1/2 in micromass culture and stimulating for 21 days (C3H10T1/2) in chondrogenic medium supplemented with BMP-2, TGFβ1 and with or without 5 µg/ml mouse recombinant S100A8. Expression of mRNA levels of MMPs were measured using RT-qPCR. The MMP mediated cartilage matrix breakdown neoepitope VDIPEN was visualized with specific rabbit anti-VDIPEN antibodies counterstained with Orange G and staining quantified using the LAS image analysis software.

Results: First, we studied the effect of S100A8/A9 on osteophyte formation in the DMM model exhibiting only low synovial involvement. Kinetic studies showed that S100A8 and S100A9 were only marginally expressed within the synovium, only evident at day 7 after induction and consistent with limited synovial thickening. At day 56, we observed no significant differences in osteophyte size between S100A9/-/- and wild-type C57Bl/6 mice in the two primary locations of osteophyte development, the medial femur and tibia (105% (Figure 1) and 136% of wild-type respectively).

In contrast, during the course of CIOA, characterized by prominent thickening of the lining layer, S100A8 and S100A9 were strongly upregulated in synovium from day 7 and remained high at days 14, 28 and 42. When CIOA was elicited in S100A9/-/- mice, we found that synovial thickening was 62% lower at day 42 (2). We next measured and quantified osteophyte and enthesophyte size on day 7, 14 and 42 of CIOA, at 11 different locations around the patella and the tibiofemoral joint, both medial and lateral. In CIOA, enthesophytes were hardly present on day 7 in both wild-type and S100A9/-/- mice. However, enthesophyte size was greatly reduced on day 21 at the cruciate ligament and on day 42 at the medial collateral ligament (42% and 7% of wild-type). In contrast, osteophytes already started to develop on day 7 of CIOA, but hardly any differences were observed between wild-type and S100A9/-/- mice. Strikingly, on day 21 and 42 osteophyte size was greatly reduced in the S100A9/-/- mice at the medial side of both tibia and femur (on day 21 33% and 44% (Figure 1), on day 42 32% and 35% of wild-type). In contrast, at the lateral side enthesophyte or osteophyte size was not reduced in the S100A9/-/- mice, which is in accordance with the fact that most pathology in the CIAO model is seen medially.

One explanation for the reduced osteophyte size in S100A9/-/- mice may be a direct effect of S100-proteins on chondrogenesis, an essential phase in the enthesophyte and osteophyte development. To investigate this, we stimulated murine C3H10T1/2 MSCs in micromass culture with 5 µg/ml S100A8 (in the presence of chondrogenic medium supplemented with BMP-2 and TGFβ1) and found a marked increase in particular MMP3 mRNA. Furthermore, histological analysis of these micromasses showed an increase in the MMP-induced cartilage breakdown epitope VDIPEN as well as a strongly altered morphology (Figure 2), indicating increased remodeling.

Discussion: S100A8/S100A9 play a crucial role in enthesophyte/ osteophyte formation during intermediate and late stages of murine collagenase-induced OA, where synovial activation is high, but not in a model where synovial activation is low (DMM). S100A8/A9 could exert this effect by regulating chondrogenesis through stimulation of chondrocytes to produce and activate
MMPs thereby remodeling the cartilage matrix more efficiently. Considering also the deleterious effect of S100A8/A9 on joint destruction in OA, targeting these alarmins during OA may be very promising.

**Significance:** To our knowledge, this is the first study showing active involvement of DAMPS S100A8/A9 in enthesophyte/osteophyte formation.

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**References:**
(1) Van der Kraan, van den Berg; Osteoarthritis and Cartilage; 2007 Mar; 15(3): 237-44
(2) van Lent *et al.* Arthritis Rheum. 2012 May; 64(5): 1466-
Figure 1: Osteophytes in CIA day 21

Osteophyte size (μm²)

- WT
- S100A9 k.o.

* indicates statistical significance compared to WT.
Figure 2: VDIPEN expression in micromass culture

Figure 2: Stimulation of C3H10T1/2 micromass cultures with 5μg/ml S100A8 causes increased remodeling during chondrogenesis and increases expression of the MMP-mediated cartilage matrix breakdown receptor VDIPEN.