10 ALARMINS S100A8 AND S100A9 STIMULATE PRODUCTION OF PRO-INFLAMMATORY CYTOKINES IN M2 MACROPHAGES WITHOUT CHANGING THEIR M2 MEMBRANE PHENOTYPE

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Background Synovial activation, which plays an important role in a subgroup of osteoarthritis (OA) patients, is regulated by M1/M2 macrophage subpopulations: pro-inflammatory M1-macrophages and regulatory M2-macrophages. Alarmins S100A8 and S100A9 are found in high concentrations in synovial fluid of rheumatoid arthritis (RA) and OA patients and are correlated to inflammation and cartilage destruction.

In the current study, the authors investigated the production of S100A8 and/or S100A9 by M1- and M2-macrophages and whether S100A8 and/or S100A9 could stimulate M2-macrophages, giving them a more pro-inflammatory character.

Methods Monocytes were isolated from blood of healthy volunteers by isolating peripheral blood mononuclear cells (PBMCs) using a Ficoll-gradient followed by isolating CD14+ monocytes using magnetic-activated cell sorting (MACS). CD14+ monocytes were differentiated into M1- or M2-macrophages by 6 day stimulation with either GM-CSF or M-CSF, respectively. mRNA levels of S100A8 and S100A9 in M1 and M2 macrophages were measured with RT-qPCR. Intracellular protein production was determined using FACS analysis and S100A8/A9 secretion was measured using an ELISA. M1 and M2 macrophages were stimulated with human recombinant S100A8 and S100A9. M1 and M2 markers (IL-1β, tumour necrosis factor α (TNFα), IL-6 and CD163, CD206 respectively) were measured using RT-qPCR, FACS and/or Luminex.

Results S100A8 and S100A9 had a significantly higher mRNA expression (65- and 2.5-fold respectively, n=10) in human monocyte derived M1-macrophages compared to M2. Also, the levels of intracellular S100A8 and S100A9 were higher in M1 than in M2 macrophages (4.3-fold and 6.6-fold higher respectively, n=6). The secreted protein level of the S100A8/A9 heterodimer was elevated from 55.5 ng/ml in M2 to 102.8 ng/ml in M1 macrophages (n=10).

Stimulation of human monocyte derived M2-macrophages for 24 h with S100A8 and S100A9 showed a marked upregulation of several pro-inflammatory markers: IL-1β expression was increased by S100A8 and S100A9 on mRNA level (33- and 16-fold, n=10). At the protein level IL-6 and TNFα were strongly upregulated by S100A8 71- and 146-fold and by S100A9 93- and 121-fold, respectively (n=6).

Interestingly however, S100A8 or S100A9 stimulation did not alter the level of M2 membrane markers CD163 and CD206, both at mRNA as well as protein level.

Conclusions Stimulation of M2 macrophages with S100A8 and S100A9, produced in high amounts by M1- macrophages, upregulates pro-inflammatory cytokines IL-1β, IL-6 and TNFα while M2 membrane markers CD163 and CD206 remain unchanged.

S100A8 and S100A9 production during OA could therefore contribute to synovial activation by stimulating the M2 macrophage towards a more pro-inflammatory phenotype.

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