Participation of cartilage in articular defense mechanisms with the production of antimicrobial peptides

Varoga D, Pufe T, Harder J, Mentlein R, Petersen W, Tillmann BN, Goldring MB, Paulsen F
Department of Anatomy, and Orthopaedic Surgery, University of Kiel, Department of Orthopaedic Surgery, New England Baptist Bone and Joint Institute, Harvard Institutes of Medicine, Boston, USA, Clinical Research Units, University of Kiel, Germany

Introduction: Antimicrobial peptides (AP) are effector molecules of the innate immune system. They display direct antimicrobial activity without any help of T- and B-cells and are responsible for a fast and effective response to invading microorganisms in the first hours after microbial colonization. Recent data suggest a multifunctional role for the AP. Beside their strong antibacterial activity they are involved in epithelial healing and neoangiogenesis, necessary processes in tissue repair after microbial colonization. Moreover AP have the capacity to link between innate and adaptive immunity (1). Recently, we have shown that articular cartilage is able to produce human beta defensins (HBD) constitutively or in response to proinflammatory cytokines like TNF-a. The aim of the present study was to evaluate the influence of gram-positive and gram-negative bacteria on the AP-expression in chondrocytes. Moreover we assessed the protein-levels of human beta defensin 2 after interleukin-1/6 and Pseudomonas aeruginosa stimulation and verified their influence in neoangiogenesis. A septic arthritis mouse model was used to demonstrate the in vivo expression pattern of mouse beta-defensin 2-4 (MBD) after Staph. aureus inoculation.

Materials and Methods: The human TC-28A2 chondrocyte monolayer culture was used for stimulation experiments. The regulation of the human beta defensins 2 and 3 after stimulation with supernatants of Staph. aureus (1:30 dilution), Pseudomonas aeruginosa (1:100 dilution) or cytokines like IL-1/6 (10ng/ml) was assessed on transcriptional level by Real-time RT-PCR and on protein level by means of immunohistochemistry and immunoblot. The influence of HBD-3 (10µg/ml) in promoting neoangiogenesis was verified with a VEGF (vascular endothelial growth factor)-Sandwich-ELISA. Antibodies against mouse beta defensin 2, 3 and 4 (Santa Cruz, CA, USA, all 1:500 dilution) were used to evaluate the articular AP-production in a septic arthritis mouse model. The injections (10µl/10³ CFU/µl Staph. aureus) were done from the frontal aspect of the murine knee joint under general anesthesia.

Results: RT-PCR revealed constitutive expression of human beta defensin 2 and 3 in TC28A2 chondrocytes. After stimulation with the proinflammatory cytokines IL-1 and IL-6, the HBD-2 expression doubles on mRNA and on protein level (Fig.1). The co-incubation of chondrocytes with Pseudomas aeruginosa led to a 30 fold induction of HBD-2 transcripts, whereas Staph. aureus failed to induce HBD-2 (Fig. 2). Immunoblot demonstrated, that chondrocytes have the ability to produce HBD-2 protein in a low µg range after 24 hours co-incubation with Pseudomonas aeruginosa (Fig. 3). In vitro studies from Harder et al. (2) found effective killing of gram-negative bacteria (E.coli, Pseudonas aeruginosa) through HBD-2 with LD 90 near 10µg/ml. A slight up-regulation was measured on transcriptional level for HBD-3 after 6 hours Staph. aureus incubation. VEGF levels were clearly increased after stimulation of the cultured chondrocytes with HBD-3 protein. The septic arthritis mouse model revealed an upregulation of mouse beta defensin 2, 3 and 4 in cartilage and subchondral bone after intraarticular inoculation of 10µl/10³ CFU/µl Staph. aureus (Fig. 4).

Discussion: This report shows previously unrecognized functions of human chondrocytes. Besides its biomechanical properties articular cartilage has the ability to release antimicrobial substances when challenged with microorganisms in vitro and in vivo. The up-regulation of HBD-2 in cartilage in low µg ranges reflect the power of articular joints to defend microbial invasion with the production of endogenous antibiotics. The increase of VEGF-expression in TC28A2 chondrocytes after stimulation with HBD-3 reflect the multifunctional role of antimicrobial peptides. However the role of antimicrobial peptides in inflammatory joint disease requires further elucidation.