INFLAMMATORY INDUCTION OF ANTIMICROBIAL PEPTIDES IN SYNOVIAL MEMBRANES

*Lippross; S; **Pufe, T; **Klostermeier, E; **Wruck, C; **Grohmann, S; **Harder, J; **Varoga, D
*Biomaterials and Tissue Engineering Program, AO Research Institute, Davos, Switzerland
**Department of Anatomy, University Hospital of Kiel, Germany
deikevaroga@hotmail.com

INTRODUCTION
Antimicrobial Peptides (AP) are the key mediators of the innate human host defense. They act as potent antimicrobial agents and chemoattractant molecules. Previous studies have demonstrated the existence and upregulation of human β-defensins within inflammatory and degenerative diseases of the human joints (1). Induction of human β-defensin 2 (hBD-2) in epithelial tissues like the skin is mediated by the activation of Toll-like receptor (TLR)-2 and -4, thus leading to nuclear translocation of transcription factors such as NF-κB and AP-1. The following study investigated the expression of hBD-2 in mesenchymal human synovial membranes and cultured human synoviocytes after stimulation with proinflammatory cytokines and bacterial culture supernatants.

METHODS
HBD-2 expression in healthy and inflamed human synovial membranes was illustrated by immunohistochemistry (IHC). The existence of TLR was examined by RT-PCR and IHC. Immortalized healthy human synoviocytes (K4IM) were used for further in vitro experiments. To examine hBD-2 expression, cultured cells were stimulated by proinflammatory cytokines (IL-1, TNF-α, 10ng/ml) or bacterial culture supernatants. Values are the mean +/- standard deviation, * = P < 0.05 versus controls.

RESULTS
The induction of hBD-2 in human synovial membranes in case of pyogenic arthritis was shown by immunohistochemistry (Fig. 1A and B). Healthy synovial membranes express Toll-like Receptors 1-5, but TLR-2 and TLR-4 were induced in inflammatory joint disease (Fig. 1). Cell culture experiments indicated transcriptional upregulation of hBD-2 after stimulation with IL-1, -6 and TNF-α or Pseudomonas aeruginosa and Staphylococcus aureus supernatants (Fig 2, 3). Transient transfection with a hBD-2 promoter construct (Fig. 4) revealed a dose dependent increased promoter activity depending on stimulation by the proinflammatory cytokine IL-1.

DISCUSSION
The present study provides the first documentation of an inducible system of potent endogenous antibiotic peptides in synovial membranes. As shown for other tissues, IL-1, TNF-α and bacteria play a key role during their activation. Future experiments will elucidate the signalling pathway using targeted disruptions in our promoter construct and investigate the role of TLR-activation within the complex innate immune system. Once assessed the regulatory pathways, iatrogenic stimulation of antimicrobial peptides may be an option in the treatment of pyogenic arthritis in future.

REFERENCES