**Introduction:** The innate immune system represents an ancient host defense mechanism. The most important effector mechanism of cell-mediated innate immunity is the production of antimicrobial peptides (AP) in response to pathogens. The AP could be released from circulating cells or could be induced in different epithelia in order to limit infections in the first hours after microbial colonization (1). Recent investigations showed the upregulation of antimicrobial peptides upon treatment with TNF-α, IL-1β or bacteria (2). So far no information about expression and regulation of antimicrobial peptides in synovial membrane are available and the existence of the previously unrecognized innate defense mechanism in human articular joints (3) is still unconfirmed.

The purpose of the study was to determine whether human synovial membrane express antimicrobial peptides and toll-like-receptors under regular conditions and to investigate potential differences in case of osteoarthritis, rheumatoid or pyogenic arthritis.

**Materials and Methods:** Healthy synovial membrane was obtained from cadavers from the Institute of Anatomy. Synovial membrane was obtained from patients who were suffering from osteoarthritis (OA), rheumatoid arthritis (RA) or pyogenic arthritis (PA) and underwent synoviectomy in the Department of orthopaedic surgery. All samples from pyogenic arthritis contained positive microbial cultures for Staphylococcus aureus. After removal, one half of each tissue was immediately frozen in liquid nitrogen and stored at –70°C. The other half was fixed in 4% formalin and embedded in paraffin until immunohistochemistry was performed. Immunohistochemical staining was done from 7µm deparaffinized sections and incubated with antibodies against secretory phospholipase A₂ (sPLA₂, 1:10, Upstate Biotechnology, Lake Placid, USA), Matriptysin (MMP7, 1:100, Chemicon Int., Temecula, USA), human neutrophil-defensins (HNP 1-3, 1:800, Bachem, Heidelberg, Germany), human beta defensin 1 and 2 (HBD 1 and 2, 1:500, Santa Cruz Biotechnology, USA), lysozyme (1:200, Dako, Glostrup, Denmark) and lactoferrin (1:150, Dako, Glostrup, Denmark). Reverse transcriptase polymerase chain reaction (RT-PCR) was performed as previously described (3) for bactericidal permeability-increasing protein (BPI), heparin-binding protein (CAP-37), human cationic antimicrobial peptide (LL-37), human alpha defensin 5 (HDS), human alpha defensin 6 (HD6), human beta defensin 1 (HBD-1), human beta defensin 2 (HBD-2), human beta defensin 3 (HBD-3) and TLR-4. Western-Blot was used to determine TLR-4 expression on untreated and on stimulated synoviocytes in-vitro. The study was approved by the ethics review board of the University of Kiel.

**Results:** Immunohistochemistry revealed lactoferrin, lysozyme, sPLA₂ and MMP7 to be present in type A synoviocytes of all samples. HNP1-3 antibody visualized a few granulocytes inside the stroma of the synovial membrane which were clearly distinguishable from type A synoviocytes in colocalization experiments with CD68. HBD-1 was detected only in some cases in type B synoviocytes. Reverse transcriptase polymerase chain reaction revealed additionally CAP-37 and HBD-1 mRNA in healthy synovial tissue. LL-37 and HBD-1 were detected in osteoarthritic and pyogenic membrana synovialis with RT-PCR. HBD-3 was upregulated in case of osteoarthritis or pyogenic arthritis. RT-PCR revealed CAP-37 expression in all inflamed synovial samples.

**Conclusion:** The human synovial membrane produces a variety of antimicrobial peptides, which are able to kill microbes by forming pores. Under inflammatory conditions the expression pattern of several antimicrobial peptides changes (Fig.2) suggesting that some AP are induced in response to the inflammatory process. This response seems to be mediated by toll-like receptors (TLR) which were present on cultured synoviocytes and were upregulated in case of inflammatory cytokines as shown by Western-Blot analysis (Fig.3) and RT-PCR. The ability of the peptides to mobilize various types of phagocytic leucocytes, immature dendritic cells and stimulation of IL-8 production provide evidence for their participation in alteration and amplifying innate and adaptive immunity and reflect their important role in the pathogenesis of inflammatory joint disease. Synthetic HBD-3 shows antimicrobial effects even to multiresistant Staph. aureus in-vitro, so it may be useful in the treatment of pyogenic arthritis in future, because Staph. aureus contributes over two-thirds of the identified microorganism. The role of the antimicrobial peptides in inflammatory joint disease awaits further elucidation.

**Fig. 1:** Detection of HBD-1 with immunohistochemistry in synovial membrane in presence of osteoarthritis.

**Fig. 2:** Detection of AP in synovial tissue by RT-PCR

**Fig. 3:**

49th Annual Meeting of the Orthopaedic Research Society
Poster #0729