INDUCTION OF ENDOGENOUS ANTIBIOTICS IN BACTERIAL BONE INFECTION

INTRODUCTION
Osteomyelitis often causes functional impairment due to tissue destruction and the incidence of this condition appears to be increasing. The advancing age of the general population with an increased incidence of musculoskeletal surgery and intake of immunomodulating drugs such as glucocorticoids or methotrexate contribute to increased numbers of bone and joint infections. Antimicrobial peptides (AP) are effectors of the innate defence system and play a key role in host protection at cellular surfaces. Some of them are produced constitutively, whereas others are induced during infection. Human beta-defensins (HBD) represent a major subclass of antimicrobial peptides and act as a first line defence through their broad spectrum of potent antimicrobial activity (1). The aim of the present in vitro and in vivo investigations was to study the expression and regulation of HBD-2 in the case of bacterial bone infection and to analyze the effects of immunosuppressive drugs on bone-derived AP-expression.

METHODS
Samples of healthy human bone, osteomyelitic bone and cultured osteoblasts (hFOB cells) were assessed for the expression of human β-defensin-2 (HBD-2) by RT-PCR, immunohistochemistry or ELISA. Regulation of HBD-2 was studied in hFOB cells and primary osteoblasts (PromoCell, Heidelberg, Germany) after exposure to bacterial supernatants of Staphylococcus aureus (SAS) or Pseudomonas aeruginosa (PAS), proinflammatory cytokines (IL-1, 10ng/ml) and immunosuppressive drugs (glucocorticoids, methotrexate) and was assayed by ELISA. An osteomyelitis mouse model was performed to demonstrate the regulation of the murine homologue of HBD-2, named MBD-3, by real time RT-PCR and immunohistochemistry. For that purpose, the murine tibiae were exposed to a clinical isolate of Staphylococcus aureus (10^6CFU/ml). 12 hours after bacterial challenge, the bones were removed and either prepared for immunohistochemistry or real-time RT-PCR analysis.

RESULTS
ELISA experiments demonstrated, that samples of infected bone produce higher levels of endogenous antibiotics such as HBD-2 when compared with samples of healthy bone (Figure 1). Systematic examination revealed the production of HBD-2 in healthy human bone, cultured primary and hFOB osteoblasts under standard conditions. After exposure of these cells to gram-positive bacteria or proinflammatory cytokines a clear induction of HBD-2 was observed as essayed by PCR and ELISA (Figure 2). Interestingly, only gram-positive bacteria such as Staphylococcus aureus were able to provoke induction of HBD-2. Additional treatment with glucocorticoids (Dexamet) or methotrexate prevented bacteria mediated AP-induction. TSB (bacterial growth medium). Values are the mean +/- standard deviation, * = P < 0.05 versus controls.

DISCUSSION
Our study demonstrates that osteoblasts are able to produce anti-inflammatory peptides such as HBD-2 in vitro and in an animal model of staphylococcal osteomyelitis. We provide evidence for a new role of osteoblasts during infection of bone tissues, namely, the ability to produce antimicrobial peptides and modulating immune responses in inflammatory bone diseases. Immunosuppressive drugs such as glucocorticoids or methotrexate may increase the susceptibility to bone infection by decreasing AP-expression levels in case of microbial challenge. Novel approaches to management are required particularly in the era of multi-resistant bacterial strains. Current investigation will focus on the regulation of human β-Defensins in bone and may allow artificial amplification for prevention of bacterial bone infection in the future.

REFERENCES
(1) Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2004