LOCALISATION OF BACTERIA DURING HEMATOGENOUS OSTEOMYELITIS

Christina Ebert1,2, Astrid Tannert1, Verena Hörr3, Carol Geppert4, Christine Pöllath2,3, Lorena Tuchscherr2,3, Bettina Löffler2,3, Jürgen Popp1,2, Ute Neugebauer1,2
1Leibniz-Institute of Photonic Technology, Albert-Einstein-Straße 9, Jena, Germany
2Center for Sepsis Control and Care, Jena University Hospital, Jena, Germany
3Institute for Medical Microbiology, Jena University Hospital, Jena, Germany
4Institute of Pathology, Universitätsklinikum Erlangen, Erlangen, Germany

E-mail: astrid.tannert@leibniz-ipht.de

KEY WORDS: intracellular S. aureus, confocal bone tissue microscopy, two photon fluorescence microscopy, osteomyelitis mouse model

Hematogenous osteomyelitis is a bone infection which may be caused as a long-term consequence of sepsis. This inflammatory disease is caused by the spread of bacteria from the bloodstream to the bone where pathogens can persist for years and finally lead to bone deformation. The most common pathogen causing this form of osteomyelitis is S. aureus. Chronic infections with this pathogen are presumably associated with adaptation to bone tissue and host cell invasion which makes them especially difficult to treat with antibiotics. Using an established mouse model of hematogenous osteomyelitis [1], we investigated the pathogenesis and especially the localisation of pathogens in the course of infection from the acute to the chronic phase. After determining pathological changes in behaviour and by X-ray, mice in the acute (1 week of infection) and chronic (6 weeks of infection) phase were sacrificed. Cryosections of isolated bones were produced after fixation and decalcification. These sections were subjected to immunofluorescence labelling of S. aureus and the bone marker osteocalcin and counter-stained with DAPI and phalloidin to reveal the shape and location of host cells. For histopathological comparison haematoxilin & eosin staining was applied to some slices. Using one- and two-photon confocal imaging, we could identify residing S. aureus in bone sections from mice with both acute and chronic osteomyelitis. Two-photon microscopy was especially useful for detecting pathogens in several μm depths of the sections. Compared to one-photon confocal microscopy, images acquired using two-photon excitation showed higher signal-to-noise ratio and contrast. Remarkably, we could detect S. aureus at quite different locations in both the acute and chronic phase of osteomyelitis ranging from the hard bone, bone marrow to associated connective tissue and attached muscle fibres. While in the acute phase no pathological transformations were detectable by X-ray, deformations were clearly visible in the chronic phase and correlated well with the fluorescence images from these sections where bacteria in clustered areas were found. Some of the pathogens, especially in the acute phase colocalised with osteocalcin, a marker of bone formation, which is produced by osteoblasts and secreted into the extracellular matrix of bone. Intracellular localisation of S.aureus could also be detected in the acute and chronic phase.

References

Acknowledgement
Financial support from the BMBF via the CSCC (FKZ01EO1502) and DGF (JBIL, FKZ PO 633/29-1, BA 1601/10-1) is highly acknowledged.