LOCALISATION OF BACTERIA DURING HEMATOGENOUS OSTEOMYELITIS

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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 adaption 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 hematoxilin & 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

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