The role of macrophages and intracellular survival of *Staphylococcus aureus* – new insights from direct evaluation of human tissue samples

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**Background**

*Staphylococcus aureus* (SA) can cause various infections and is associated with high morbidity and mortality rates of up to 40%. Antibiotic treatment often fails to eradicate SA infections even if the causative strain has been tested susceptible *in vitro*. The mechanisms leading to this persistence is still largely unknown. In our work, we hypothesize that interactions with host cells allow SA to persist at the site of infection.

**Methods**

We established a sampling workflow to receive tissue samples from patients requiring surgical debridement due to SA bone-and joint or soft-tissue infections. We developed a multiplex immunofluorescent staining protocol which allowed us to stain for SA, leukocytes, neutrophils, macrophages, B-cells, T-cells, DAPI and cytoplasmatic marker on the same sample slide. Further, distance of SA to cell nuclei was measured. Interaction of immune cells and SA on a single cell level was investigated with high-resolution 3D microscopy. We then validated our findings applying fluorescence-activated cell sorting (FACS) on digested patient samples. Finally, we aimed to reproduce our *ex vivo* patient results in an *in vitro* co-culture model of primary macrophages and clinical SA strains, where we used live cell microscopy and high-resolution microscopy to visualize SA-immune cell interactions and a gentamicin protection assay to assess viability of SA.

**Results**

Here, we revealed that CD68+ macrophages were the immune cells closest to SA with a mean distance of 56μm (SD=36.4μm). Counting the amount of SA, we found in total >7000 single SA in nine patients. Two-thirds of SA were located intracellularly. Two-thirds of the affected immune cells with intracellular SA were macrophages. The distribution of intra- to extracellular SA was independent of ongoing antibiotic therapy and underlying infection type. FACS confirmed these findings. In our co-culture model, intracellular SA remained alive for the whole observation period of eight hours and resided in RAB5+ early phagosomes.

**Conclusion**

Our study suggests an essential role of intracellular survival in macrophages in SA infections. These findings may have major implication for future treatment strategies.