Macrophage visualization in *S. aureus* infection of zebrafish

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In *Staphylococcus aureus* infection, antibiotic treatment failure in the absence of genetically encoded resistance (antibiotic tolerance) remains a problem despite many available antibiotics. Host innate immune cell activities can create an environment that induces antibiotic tolerance in the bacteria. This project aims to establish a model of *S. aureus* infection in zebrafish (*Danio rerio*) that enables the visualization of the innate immune response to bacterial infection. Ultimately, we hope to use this model to probe how the innate immune response contributes to antibiotic tolerance in *S. aureus*. We used genetically modified larval zebrafish with a dual-macrophage-labeling system: a BFP reporter marking all macrophages and an inducible GFP which is upregulated upon macrophage activation. We infected these zebrafish larvae by microinjecting mCherry- or dsRed-labelled *S. aureus* in the brain and used confocal microscopy to visualize macrophage-bacterial interactions. We also measured bacterial burden to map infection dynamics. Our results indicate that macrophages phagocytose *S. aureus* and are able to prevent bacterial population growth. Activated macrophages containing *S. aureus* aggregate, and antibiotics fail to kill the bacteria. These results indicate a successful infection model that we can leverage in future studies of *S. aureus* host-pathogen interaction and antibiotic tolerance.

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