TARGETING STS TO OPTIMIZE HOST ANTIMICROBIAL IMMUNITY

Specific Aims

Persistent infectious diseases caused by bacterial and fungal pathogens are leading contributors to the global disease burden (1). Unfortunately, many current antibiotics suffer from a number of drawbacks, including toxicity, limited bioavailability, and a narrow spectrum of activity. Further, a lack of effective vaccine strategies and the appearance of antibiotic resistant strains contributes to high mortality (2-5). Improving overall treatment strategies is an urgent priority.

We and others have established two homologous phosphatases, Sts-1 and -2, as negative regulators of immune signaling pathways (6). Importantly, mice lacking Sts expression (*Sts*-/-) are profoundly resistant to lethal infection by a number of virulent microbial pathogens, among them the deadly bacterial pathogen *Staphylococcus aureus* (7-9). Resistance is associated with rapid pathogen clearance, reduced inflammation, and significantly increased survival. Interestingly, *Sts*-/- macrophages also show accelerated clearance of internalized *S. aureus* following *ex vivo* infection, with 24 hr CFUs being several orders of magnitude lower in *Sts*-/- cells. Although the role of Sts in controlling immune response pathways is unknown, the favorable outcome associated with Sts inactivation reveals the existence of an important regulatory pathway that may be manipulated to potentiate the host response to pathogen infection.

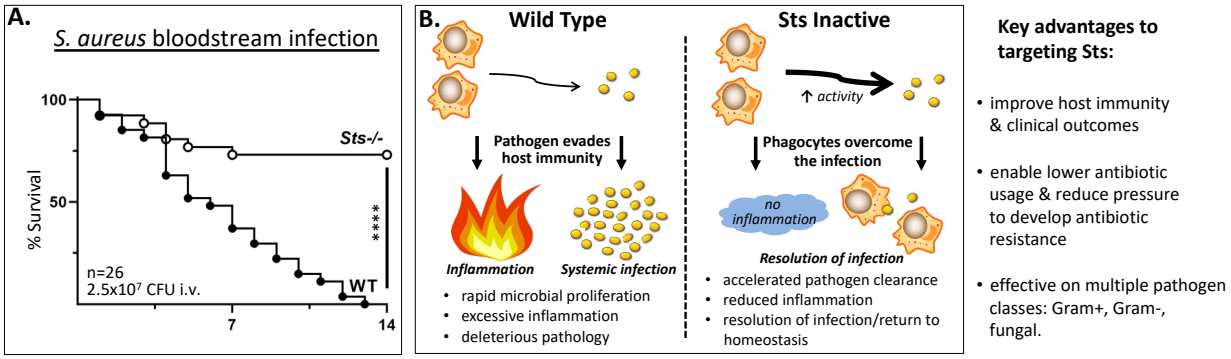

Figure 1: (A) Resistance of *Sts*-/- mice to lethal bloodstream infection by *Staphylococcus aureus*. **(B)** Sts inactivation potentiates the antimicrobial properties of host phagocytes, tipping the balance of interaction between host and phagocytes in favor of the host.

Further understanding of the role of Sts in regulating host immunity is essential to establishing them as viable therapeutic targets. Therefore, in **Aim 1** we define the role of Sts in regulating transcriptional responses within infected macrophages. In **Aim 2**, we seek to improve the drug-like properties of Sts-242, a recently discovered small molecule Sts inhibitor. Together, these Aims will strengthen and extend our current preliminary data to allow for successful development of a drug discovery R01 centered on developing a new host-directed anti-microbial treatment strategy.

Aim 1. Determine how Sts regulates the dynamic interaction between macrophages and the bacterial pathogen *Staphylococcus aureus.* Very little is known regarding the role of Sts in controlling immune response pathways critical for phagocyte-mediated *S. aureus* clearance. We will perform dual RNASeq analysis to determine how Sts regulates macrophage antimicrobial gene expression patterns and how *S. aureus* responds at the transcriptional level to the uniquely restrictive intracellular environment within *Sts*-/- macrophages. These studies will provide critical information with which to develop new, testable hypotheses regarding the role of Sts in regulating macrophage pathways and effector responses.

Aim 2. Perform a structure-activity relationship (SAR) analysis of Sts-242. Sts-242 has modest biological activity and limited solubility. We will improve the drug-like properties of Sts-242 by generating and testing a ~100 compound library of analogs. This will yield critical information for the design and synthesis of lead-like compounds that will serve as the foundation of an R01 proposal whose goal will be to develop immune-enhancing drugs to reduce the morbidity and mortality associated with serious microbial infections involving pathogens such as *Staphylococcus aureus*.