ABSTRACT

Background and Objective: The Staphylococcus aureus (S. aureus) exoprotein secretion system (Saee/R) is a two-component protein system within Staphylococcus aureus that has been linked to this pathogen’s ability to survive within human neutrophils (polymorphonuclear leukocytes or PMNs). Prior studies have shown that an extracellular (EC) loop—consisting of nine amino acid residues on SaeeS, is vital for S. aureus to sense and respond to extracellular stimuli—specifically components of human PMNs. Additionally, γ-hemolysin (hlgA) is a predominant virulence factor that targets immune and red blood cells. This toxin has been shown to be regulated by SaeeR/S. New hlgA-GFP S. aureus cell strains—including point mutations of the residues on the EC loop—have been developed in order to study the role of each residue in S. aureus survival. All strains contained a plasmid on which the hlgA gene was linked to the GFP reporter. The current study sought to both characterize the activity of these strains in the presence of human PMNs as well as determine if hlgA-GFP fluorescence was a legitimate proxy for measuring hlgA expression.

METHODS: We investigated the expression of hlgA following neutrophil phagocytosis of S. aureus using hlgA-GFP reporter strains. Flow cytometry was used to measure GFP fluorescence within samples after being incubated for varying lengths of time.

RESULTS: Our findings suggest that the hlgA-GFP reporter can be used to show hlgA expression at later time points (4-6 hr). However, at earlier time points (0.5-2 hr) the hlgA-GFP reporter was not sensitive enough to assess hlgA transcription. This is likely because GFP was not present in high enough quantities to be detected.

Figure 1. A scanning electron micrograph of a human neutrophil interacting with Staphylococcus aureus (green). Previous studies have shown that the ability of S. aureus to sense the neutrophil dictates outcome of infection.

Figure 2. The nine residue EC loop of SaeeS. Previous studies have identified this loop as the sensing domain of SaeeS.

Figure 3. A schematic of the experimental setup used to measure hlgA expression.

RESULTS CONTINUED

hlgA-GFP assay: Cultures were grown to OD 1.5 (at 600 nm). The resuspended cultures were then synchronized with neutrophils in a 96 well plate. The plates were incubated at 37°C with CO2. GFP fluorescence was measured on the flow cytometer.

Growth Curves: To determine proper PMN to bacteria ratio growth curves were conducted. Cultures were grown for 6 hours. Samples were taken every hour—the optical density (OD) was measured and samples were plated on TSA plates. Colonies were counted the following day.

Figure 4. Growth curves of four hlgA-GFP S. aureus strains. A. Colony forming units (CFUs) per mL culture. These results verified that no strains have a greater select. B. Optical Density (OD) as measured at 600 nm. A & B provide a means for determining bacterial concentration of specific cultures.


Figure 6. PMN overlay showing GFP shift in fluorescence over time of the hlgA-GFP reporter. A. PMNs only. B. WT saeeS. C. ΔsaeeS. D. Complement ΔsaeeS + WT. E. M31A. F. W32A. G. F33A. H. N34A.

Figure 7. Change in GFP fluorescence over time. Results are shown as the geometric mean of fluorescence. By 6 hours, all strains exhibit increases in fluorescence.

CONCLUSIONS/DISCUSSION

• The hlgA-GFP reporter is a viable proxy for hlgA expression.
• The reporter is not sensitive enough at early time points (0.5-2 hr) to be accurately measured on the flow cytometer.
• The knockout strain ΔsaeeS and point mutant strains also demonstrate a shift in fluorescence at 6 hours.
• It is probable that an alternate system within S. aureus regulates hlgA at time points greater than four hours.

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