

Supplemental Information for:

A Role for Streptococcal Collagen-Like Protein 1 (Scl-1) in M1T1 Group A *Streptococcus* Resistance to Neutrophil Extracellular Traps

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Running Title: Scl-1 Protects GAS From Neutrophil Extracellular Traps

Supplemental Methods

Immunofluorescence assays

Bacterial cultures were grown to log phase and seeded on coverslips in 24-well plates for 30 min at 37°C. Bacteria were fixed with 4% paraformaldehyde and incubated in 1mg/mL lysozyme in block buffer (10% heat-inactivated fetal equine serum and 0.1% BSA in 20 mM HEPES, 140 mM NaCl, 5 mM CaCl₂, 2.5 mM MgCl₂, pH 7.4). Bacteria were subsequently incubated with 0.005 mg/mL affinity purified anti-Scl-1 or pre-immune rabbit serum (kind gifts of Magnus Rasmussen), followed by incubation with goat anti-rabbit Alexa 488 antibody (Invitrogen). Bacteria were imaged on an inverted Leica TCS SP5 confocal microscope using a 63x/1.40 oil objective and 6x digital zoom at calibrated magnifications and recorded with LAS AF software (Leica). Presence of bacteria in the field of view were verified by brightfield images (data not shown). Linear adjustments to the entire image brightness were applied uniformly for all images.

Hyaluronic acid capsule assay

Hyaluronic acid capsule from GAS was quantified as previously described (1). Briefly, bacterial cultures were grown to log phase and resuspended in water. Capsule was extracted with chloroform in Mini-BeadBeater-8 (Biospec Products). Following centrifugation, the hyaluronic acid content in the aqueous phase was determined by the hyaluronic acid test kit (Corgenix) according to the manufacturer's instructions. The absorbance was measured with SpectraMax M3 plate reader at 450 nm using SoftMax Pro software.

Fibrinogen-binding assay

Fibrinogen-binding of GAS was measured as previously described (2). Briefly, human plasminogen-depleted fibrinogen (Calbiochem) was labeled with FITC using the FluoReporter protein labeling kit (Invitrogen) and added at 100 µg/mL to GAS strains grown to log phase. Samples were incubated for 30 min with rotating and washed with PBS prior to flow cytometric analysis. Bacteria were gated by forward and side scatter, and the fluorescence intensity was measured for a total of 50,000 bacteria. Unlabeled bacteria were used as a negative control. Flow cytometry data were analyzed with FlowJo v. 9.4.10 (Tree Star, Inc.).

SpeB activity assays

Bacterial colonies were picked and grown in THB in a 96-well plate O/N at 37°C. Plates were spun to pellet bacteria, and supernatant was transferred to a new plate to an equivalent volume of activation buffer (0.1 M sodium acetate, 0.1 M acetic acid, 1 mM EDTA and 20 mM DTT, pH 5) and incubated for 30 min at 40°C. An equal volume of 2% azocasein (Sigma) in activation buffer was added to supernatants and incubated for an additional 1 h at 40°C. The absorbance was measured with SpectraMax M3 plate reader at 460 nm using SoftMax Pro software.

Whole blood killing assays

Bacteria were diluted to a final inoculum of 10^4 CFU, added to 300 µL freshly drawn blood, and rotated at 37°C. At 3 h post-infection, an aliquot of blood was removed, blood cells were lysed with water, and bacteria were enumerated on THA plates. Survival was calculated as the percentage of the initial inoculum, and due to large donor-dependent variability, results were compared with the wild-type strain.

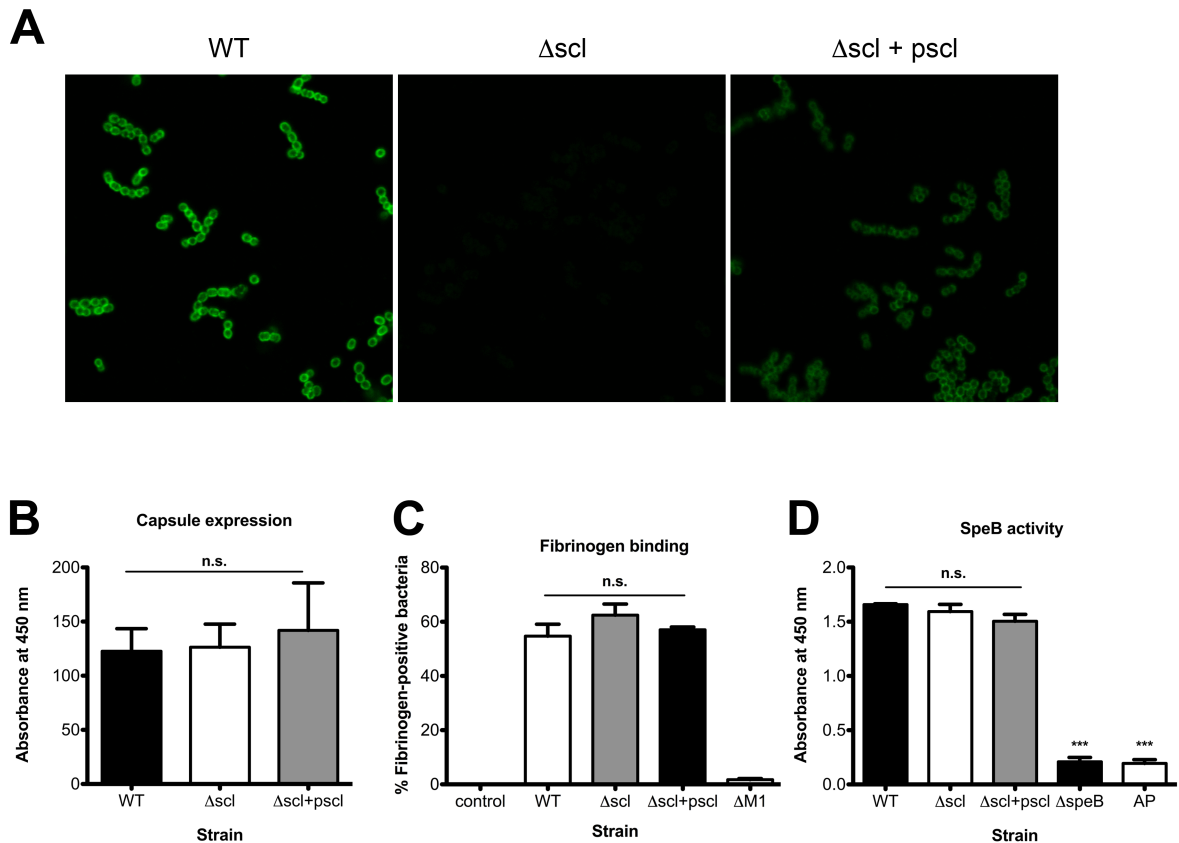


Figure S1: The Δ scl strain differs only in surface expression of Scl-1.

(A) Indirect immunofluorescence analysis of WT, Δ scl mutant and complemented strains using an affinity-purified Scl-1 rabbit antiserum. Representative images are shown. (B-D) Expression of other GAS proteins were unaffected by the deletion of *scl-1*. Surface expression of capsule (B) and M1 protein (as assessed by fibrinogen binding, C) and SpeB activity (D) are not significantly different among the WT, Δ scl mutant and complemented strains. For panels B-D, data from two independent experiments were combined, and the results are given in average \pm SEM and analyzed by one-way ANOVA with Tukey's multiple post-test (n.s., not significant). Δ M1 (C), Δ speB and AP (D) strains were included as controls.



Fig S2: Sci-1 enhances survival in whole blood.

The survival of WT, Δ scl and complemented strains in total killing assays was analyzed using whole human blood. Data were normalized to the survival of the WT strain to account for donor-dependent differences, and data from at least three independent experiments were combined. Results are given in average \pm SEM and analyzed by one-way ANOVA with Tukey's multiple post-test (**P<0.001).

Supplemental References

1. **Hollands A, Pence MA, Timmer AM, Osvath SR, Turnbull L, Whitchurch CB, Walker MJ, Nizet V.** 2010. Genetic switch to hypervirulence reduces colonization phenotypes of the globally disseminated Group A *Streptococcus* M1T1 clone. *J Infect Dis* **202**:11-19.
2. **Anderson EL, Cole JN, Olson J, Ryba B, Ghosh P, Nizet V.** 2014. The fibrinogen-binding M1 protein reduces pharyngeal cell adherence and colonization phenotypes of M1T1 Group A *Streptococcus*. *J Biol Chem* **289**:3539-3546.