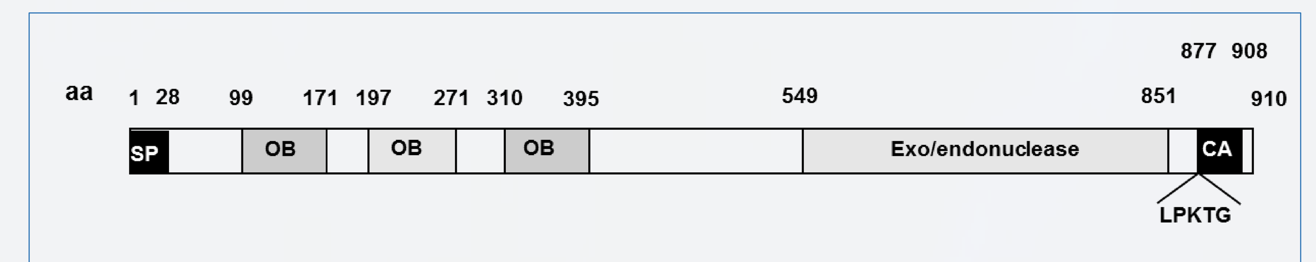
Streptococcus pyogenes nuclease A (SpnA) mediated virulence does not exclusively depend on nuclease activity

Callum Chalmers, **Adrina Hema J-Khemlani**, Chae Ryeong Sohn, Jacelyn Loh, Catherine Tsai and Thomas Proft

Department of Molecular Medicine and Pathology, School of Medical Sciences and Maurice Wilkins Centre, The University of Auckland, New Zealand

INTRODUCTION

- *Streptococcus pyogenes*, also known as Group A Streptococcus (GAS) is a major human pathogen that is the cause of diseases such as pharyngitis, tonsillitis, necrotising fasciitis, rheumatic fever and toxic shock syndrome.[1]
- *Streptococcus pyogenes* nuclease A (SpnA) is a Mg^{2+}/Ca^{2+}-dependent cell wall-anchored nuclease shown to confer GAS virulence in a mouse infection model[2].
- SpnA is able to cleave single stranded DNA, double stranded DNA, RNA, and the DNA framework of neutrophil extracellular traps (NETs).[3]



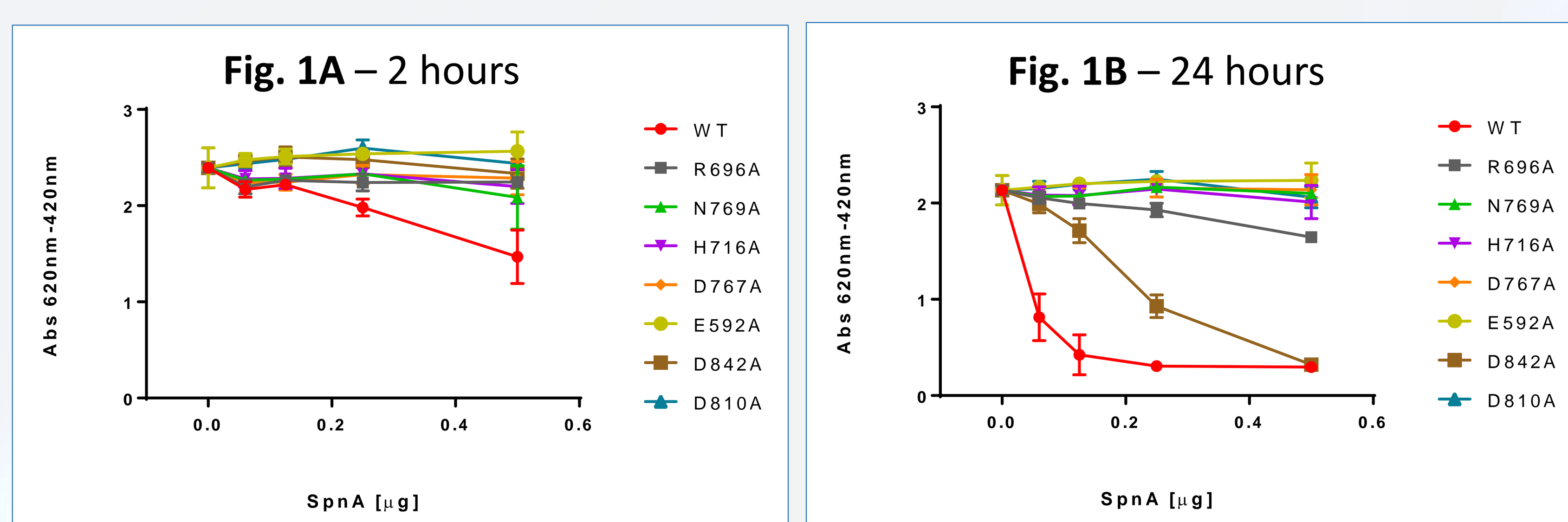
- SpnA consists of a C-terminal 302-aa exo/endonuclease domain with a cell wall-anchor (CWA) domain and a 526-aa N-terminal domain of unknown function.

The **AIM** of this study is elucidate the nuclease activity of SpnA and its role in SpnA-mediated virulence using the *Galleria mellonella* (wax worm) infection model.

RESULTS

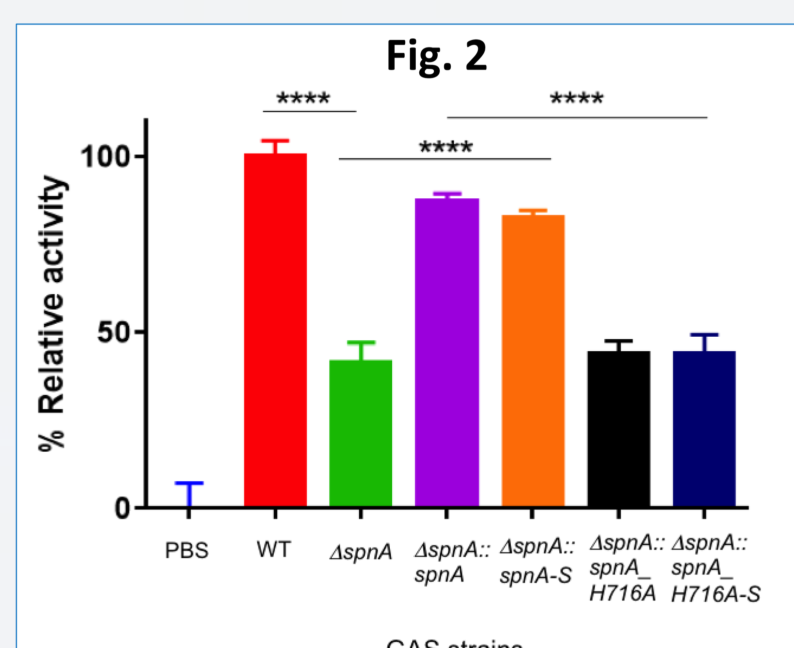
1. Mutation analysis of recombinant SpnA

- The SpnA exo/endonuclease domain was modelled onto the complete crystal structure of BP DNase I.
- Several conserved residues with predicted functions in enzymatic activity were identified, including catalytic site residues **His716**, **Asp767** and **Asp810**, predicted Mg^{2+}-binding residues **Glu592** and **Asp842**, and two potential substrate binding site residues **Arg696** and **Asn769**.
- These SpnA residues were converted to alanine and recombinant forms, along with the wild-type (**WT**), were expressed in *E. coli* and purified by immobilised metal-chelate affinity chromatography (IMAC) using Ni^{2+}-NTA.
- The nuclease activity against salmon testes DNA was determined at various concentrations of rSpnA using the methyl green assay.
- All mutants showed reduced DNAse activity after 2h (Fig. 1A) and 24h (Fig. 1B).



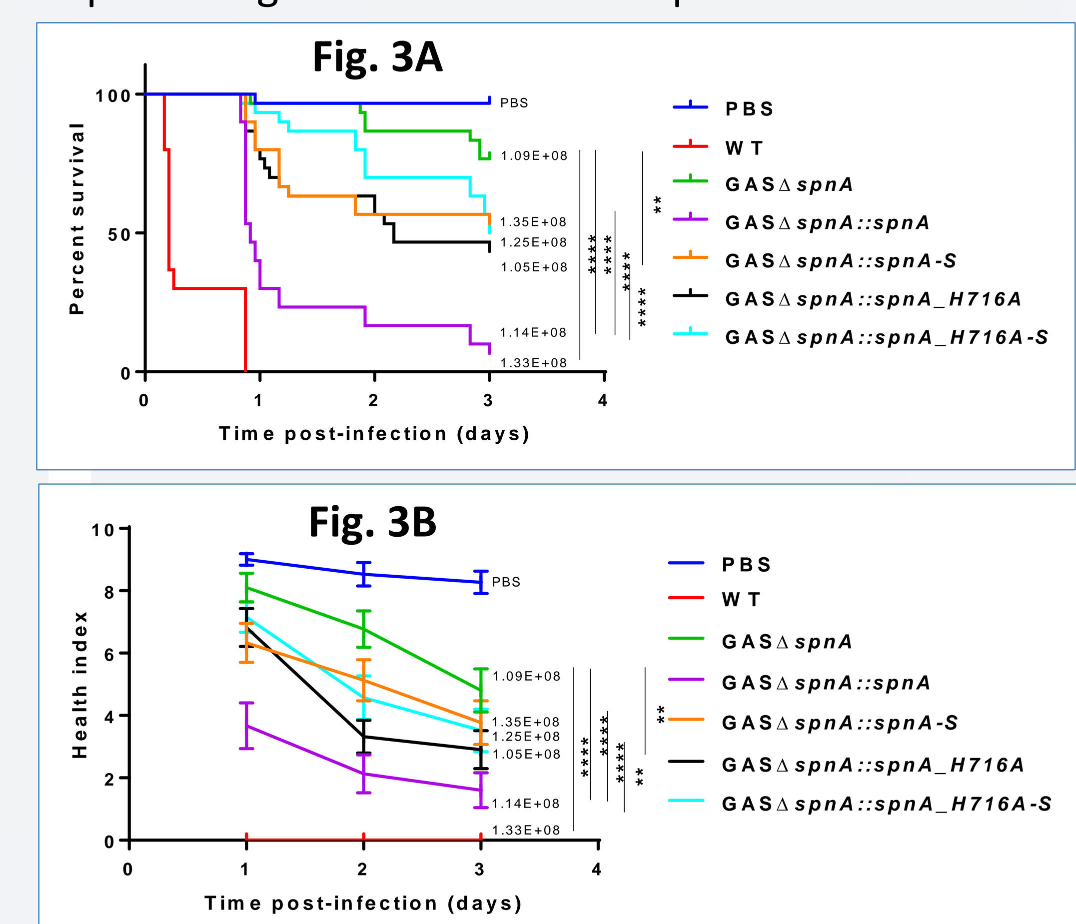
2. Generation of GAS SpnA deletion and complementation mutants

- GAS ΔspnA was generated and complemented with either wt *spnA* gene (GAS *ΔspnA::spnA*) or a nuclease-deficient *spnA* gene (GAS *ΔspnA::spnA*-H716A).
- Complemented strains either expressed the cell wall-anchored (CWA) version or a secreted (S) version lacking the CWA domain.
- The nuclease activity against salmon testes DNA was determined using the methyl green assay. Statistical analysis was performed with a one-way ANOVA test **** p<0.001.
- The H716A mutant showed comparable DNase activity with the SpnA deletion mutant
- No significant differences were observed between the CW-anchored and secreted versions.



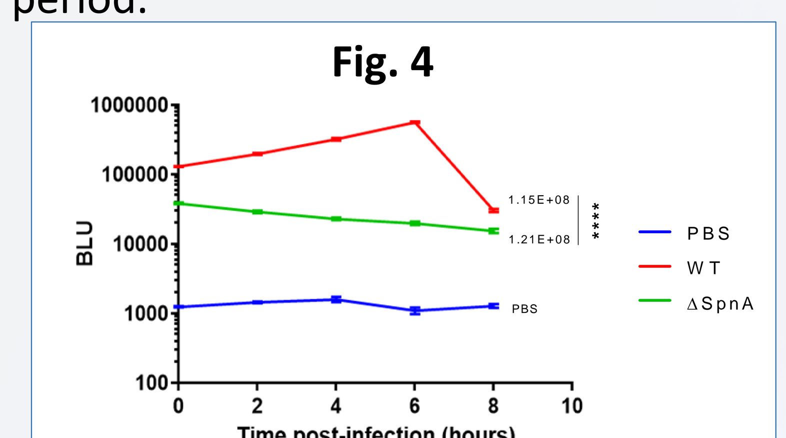
3. In vivo analysis of WT GAS and GAS mutants using the *G. mellonella* infection model.

- Groups of 10 larvae were used in 3 independent experiments. Shown are Kaplan-Meier survival curves (Fig. 3A) and mean ±SEM health index scores (Fig. 3B) of *G. mellonella* (wax worm) larvae infected with indicated doses (CFU) of WT GAS and GAS mutants over 3 days. Statistical analysis was performed with a log-rank test (A) or a two-way ANOVA test (B). ** P<0.01; **** P<0.0001.
- Infection with GAS *ΔspnA* significantly increased larval survival compared to WT GAS.
- Complementation with *spnA* (GAS *ΔspnA::spnA*) significantly decreased larval survival, but not to WT level, which is consistent with DNase activity (Fig. 2).
- The GAS *ΔspnA::spnA*-H716A mutant shows significantly greater virulence compared with the non-complemented *spnA* deletion mutant, although with lower virulence compared to WT GAS.
- GAS expressing the soluble (S) versions of SpnA and SpnA_H716A are less virulent compared to GAS producing cell wall-anchored SpnA.



4. Biophotonic imaging of infected *G. mellonella* larvae

- *G. mellonella* larvae (n=50) were infected with WT GAS or GAS *ΔspnA* expressing firefly luciferase and monitored over a 8h period.
- Larvae infected with GAS *ΔspnA* showed consistently decreasing bioluminescence after infection indicating bacterial killing (Fig. 4).
- In contrast, WT GAS increased during the first 6 hours post-infection before showing a sharp drop.



CONCLUSION

- Several predicted sites crucial for enzymatic activity of SpnA were experimentally confirmed.
- The observed virulence of the GAS *ΔspnA::spnA*-H716A mutant in *G. mellonella* larvae suggests that nuclease activity of SpnA is important for GAS virulence, but not sufficient.
- The CW-anchored version of SpnA is more virulent than the secreted version possibly due to higher local concentrations of SpnA and a more efficient destruction of NETs that surround the bacteria.
- Larval killing appears to be associated with bacterial proliferation possibly due to immune evasion mechanism.

REFERENCES

1. Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 2000; 13:470-511.
2. Hasegawa T, Minami M, Okamoto A, Tatsuno I, Isaka M, Ohta M. Characterization of a virulence-associated and cell-wall-located DNase of *Streptococcus pyogenes*. Microbiology 2010; 156:184-90
3. Chang A, Khemlani A, Kang H, Proft T. Functional analysis of *Streptococcus pyogenes* nuclease A (SpnA), a novel group A streptococcal virulence factor. Mol Microbiol 2011; 79:1629-42.00