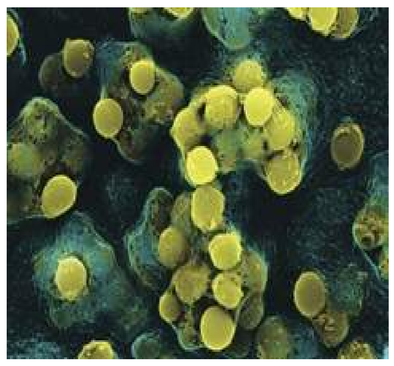
Investigations into a novel hypothetical virulence factor from Group A streptococcus



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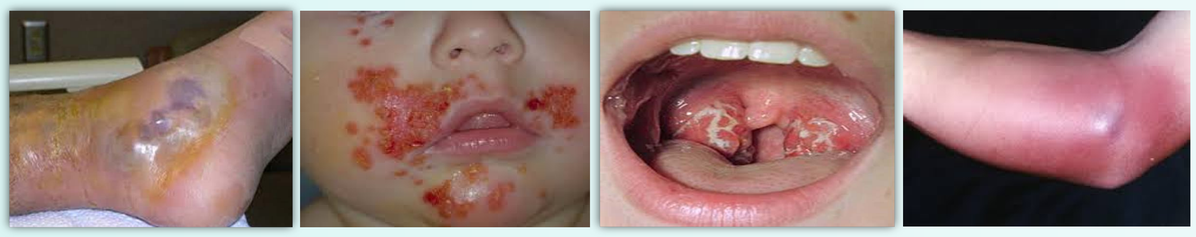
MEDICAL AND HEALTH SCIENCES

Introduction

Group A Streptococcus (GAS) or *Streptococcus pyogenes* is a major human pathogen that causes a range of diseases, from minor skin and throat infections such as impetigo and pharyngitis, to severe invasive infections such as streptococcal toxic shock syndrome and necrotising fasciitis. This is facilitated by a large arsenal of virulence factors that contribute to colonisation of host tissue, invasive spread and immune evasion. Since the completion of the first GAS genome sequencing project in 2001, the number of novel virulence factors is constantly growing. We have identified an open reading frame encoding a hypothetical 221 amino acid protein that is predicted to be secreted by the bacteria. The Spy0136 protein is highly conserved in GAS, but not found in related species, suggesting a more specialised function. The overall goal of this study is to characterise Spy0136 and determine its function. This project will also analyse the suitability of the putative virulence factor as a target for therapeutic intervention or vaccine development.

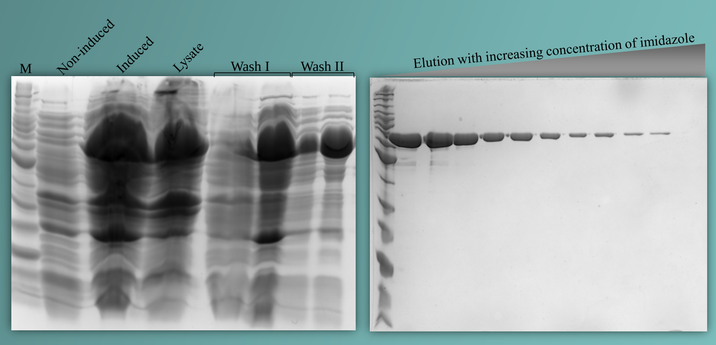
Spy0136 protein structure



Methods and Results

Expression and purification of MBP-Spy0136

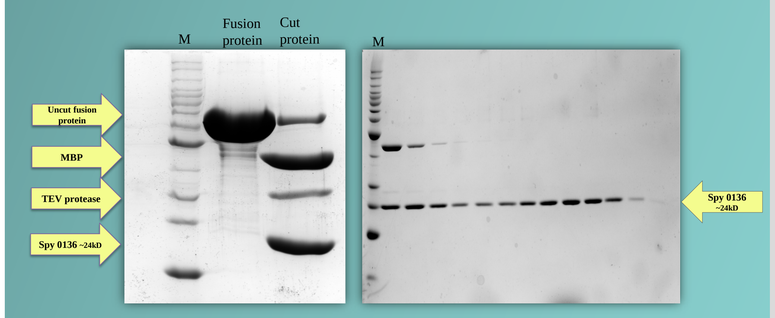
Spy0136 protein fused to Maltose Binding Protein (MBP) with a N-terminal $(His)_6$-tag from pPROEX HTa vector was produced in *E.coli*, and purified by immobilised metal-chelate affinity chromatography (IMAC) using Ni^{2+}-NTA



SDS-PAGE gel images showing expression and purification of Spy0136

Removing MBP and further purification of Spy0136 with size exclusion chromatography

Purified fusion protein was cut by rTEV protease. Spy0136 protein was separated by size exclusion chromatography

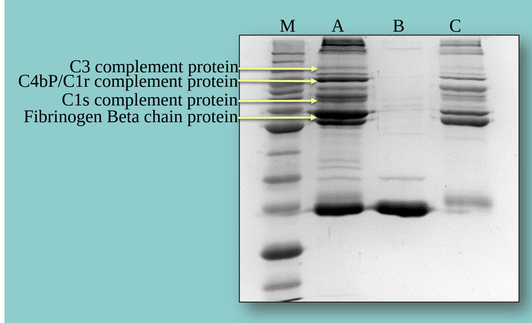


MBP-Spy0136 fusion protein after purification was treated with rTEV protease

Further purification of Spy0136 by size exclusion chromatography

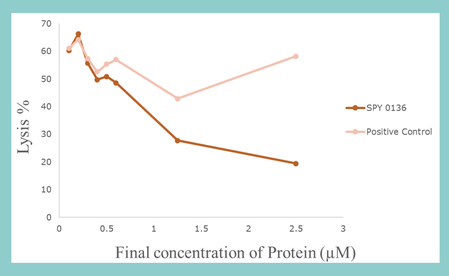
Fishing for binding partners to Spy0136 by Pull-down Assay

The purified GAS protein was used as bait in a pull-down experiment with human plasma. Several proteins were found to bind specifically to Spy0136, and were identified by mass spectrometry



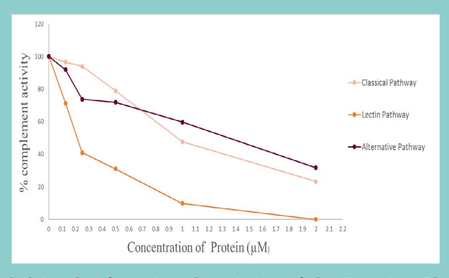
SDS-PAGE gel showing pulled down proteins
(A) rSpy0136-CNBr-Sepharose + plasma (B) rSpy0136-CNBr-Sepharose (C) inactivated CNBr-Sepharose + plasma

Evaluation of Spy0136 interaction with complement components by CH50 Assay



Spy0136 inhibits the lysis of sensitised sheep red blood cells indicating interference with classical complement pathway

Evaluation of the functional activity of Spy0136 on the three pathways of complement activation by Wieslab screenkit

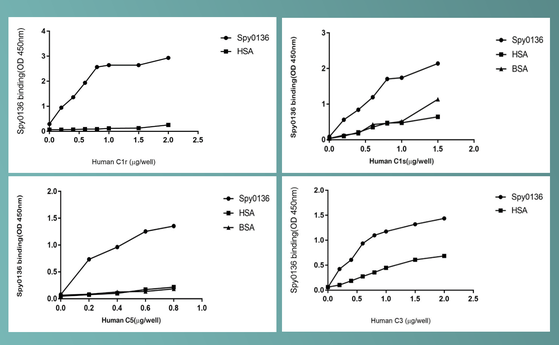


Spy0136 inhibits the functional activity of the Lectin, Classical and Alternative pathways of complement in a dose-dependent manner

Investigating the interaction of complement proteins with Spy0136 by ELISA

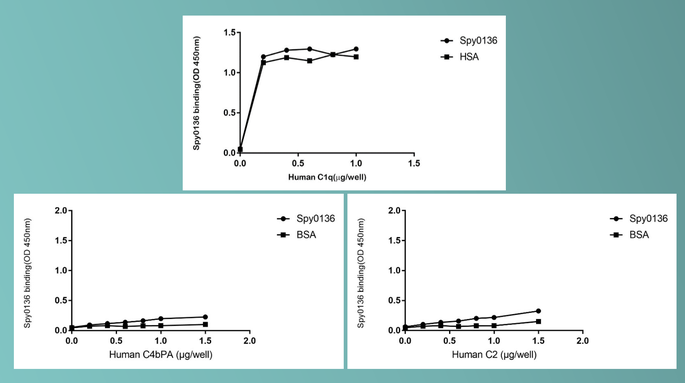
C1r, C1s, C3 and C5 Complement proteins + Spy0136

The Spy0136 interacts with four complement proteins in a dose-dependent manner



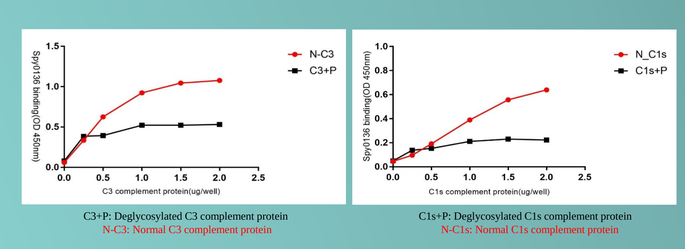
C1q, C2, C4bPA Complement proteins + Spy0136

The Spy0136 did not show interaction with the following three complement proteins



Analyzing the role of oligosaccharide residues of complement proteins in interaction with spy0136

Interaction of C3 and C1s complement proteins after deglycosylation by PNGase F enzyme were evaluated by ELISA



C3+P: Deglycosylated C3 complement protein
N-C3: Normal C3 complement protein

C1s+P: Deglycosylated C1s complement protein
N-C1s: Normal C1s complement protein

Deglycosylated complement proteins have reduced interactions with Spy0136 suggesting that the interaction between these proteins is dependent on the presence of oligosaccharide residues

Conclusion and Future Directions

Spy0136 from GAS can be produced as a soluble recombinant protein which interacts with human plasma proteins and inhibits complement-mediated lysis. It dramatically reduced the activity of all three complement pathways in a dose-dependent manner. The purified GAS protein binds to specific human complement proteins via oligosaccharides residues.
A Spy0136 deletion mutant, complementation and alanine-conversion mutants are currently being investigated for further elucidating the role of this virulence factor in GAS pathogenesis. It may be an important immuno-modulatory virulence factor and putative candidate for therapeutic intervention or vaccine development.