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Characterisation of cell wall proteins, virulence factor maturation and invasive disease trigger of Group A Steptococcus

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Characterisation of Cell Wall Proteins, Virulence Factor Maturation and Invasive Disease Trigger of Group A *Streptococcus*

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DECLARATION

This thesis is submitted in accordance with the rules and regulations of the University of Wollongong in fulfilment of the degree of Doctor of Philosophy (PhD). It does not include any material previously published by another person, except where due reference is made in the text. The experimental work described in this thesis is original work and has not been submitted for a degree in any University.

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Jason Cole

May 11, 2006

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ABSTRACT

Streptococcus pyogenes (group A *Streptococcus*; GAS) is a Gram-positive human pathogen responsible for numerous life-threatening diseases, including necrotising fasciitis and streptococcal toxic shock syndrome (STSS). The non-suppurative sequelae of acute rhematic fever (ARF) and acute post-streptococcal glomerulonephritis (APSGN) may develop upon repeated exposure to GAS. Over the last two decades, there has been a world-wide resurgence in GAS infection. In this study, a proteomic analysis was undertaken to identify the major cell wall-associated proteins of GAS. Mutanolysin cell wall extracts from GAS strain NS931 (serotype M69), NS13 (serotype M53) and S43 (serotype M6) were separated by two-dimensional gel electrophoresis and the landmark proteins identified by matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry. A total of 155 protein spots representing 74 distinct cell wall-associated proteins were identified, with molecular masses ranging from 14.4 to 77.5-kDa and a pI range of 4.4 to 7.9. Of these 74 proteins, nine putative virulence determinants (including M protein, SpeB, SpeM, FcrA, arginine deiminase, superoxide dismutase, C3-degrading protease and the two plasminogen-binding proteins SEN and Plr/GAPDH), eight glycolytic enzymes, eight carbohydrate metabolism enzymes, two chaperonins and four proteins with transporter function were detected in one or more of the GAS strains examined. Two-dimensional immunoblot analysis with pooled GAS-reactive human antisera revealed that thirty-three of these proteins were immunoreactive. Biotinylation of the GAS cell surface prior to two-dimensional western blotting identified a subset of twenty-three cell wall-associated proteins that are surface-exposed. These data illustrate the usefulness of proteomics in analysing the cell surface topology of GAS, which lays the

foundation for future work identifying new GAS virulence determinants and vaccine antigens.

The multifunctional chaperone and serine protease HtrA (DegP) is involved in the refolding and degradation of aberrant proteins destined for secretion. In numerous pathogenic organisms HtrA is required for thermostability, resistance to oxidative stress and full virulence. The HtrA of human pathogen *S. pyogenes* influences the maturation of secreted virulence factors streptolysin S and streptococcal pyrogenic exotoxin B (SpeB), and is localised at the ExPortal secretory microdomain. In this study, we examined the role of HtrA in regulating expression of GAS virulence phenotypes. An in-frame *htrA* deletion mutant displayed similar growth kinetics to the wild-type (wt) in the presence of paraquat-induced oxidative stress. Lack of HtrA did not influence capsule production or GAS adherence and invasion of human epithelial cells. Two-dimensional gel electrophoresis detected elevated levels of cleaved M protein fragments in the *htrA* mutant culture supernatant. The HtrA-mutant also had higher levels of cell wall-associated M protein than the wt. Time-course analysis of culture supernatant showed that the degradation rate of several virulence factors, including M protein, streptokinase, streptococcal enolase, superoxide dismutase A and glyceraldehyde-3-phosphate dehydrogenase, was reduced in the *htrA* mutant. This degradation corresponded to a lag in the processing of the 40-kDa SpeB zymogen to the active 28-kDa form. *In vitro* protease assays demonstrated that proteolytically active HtrA was unable to process SpeB zymogen to the active protease. These data indicate that HtrA is essential for the efficient maturation of SpeB zymogen into the active protease and the subsequent SpeB-mediated degradation of surface and secreted GAS virulence factors.

The globally disseminated *S. pyogenes* M1T1 clone causes a number of highly invasive human diseases. The transition from local to systemic infection occurs by an unknown mechanism; however invasive M1T1 clinical isolates are known to express significantly less of the cysteine protease SpeB than M1T1 isolates from local infections. Here, we show that in comparison to the M1T1 strain 5448, the isogenic mutant Δ*speB* accumulated 75-fold more human plasmin activity on the bacterial surface following incubation in human plasma. SpeB was shown to degrade several bacterial and host molecules that contribute to the accumulation of bacterial surface plasmin activity. Human plasminogen was an absolute requirement for M1T1 strain 5448 virulence following subcutaneous infection of humanised plasminogen transgenic mice. *S. pyogenes* M1T1 isolates from the blood of infected humanised plasminogen transgenic mice expressed reduced levels of SpeB in comparison with the parental 5448 used as inoculum. The M1T1 mutant Δ*speB* displayed markedly reduced virulence in this transgenic model, demonstrating a requirement for SpeB at the site of infection. We propose that the human plasminogen system plays a critical role in group A streptococcal M1T1 systemic disease initiation. Although SpeB is required for *S. pyogenes* M1T1 survival at the site of local infection, it also disrupts the interaction of *S. pyogenes* M1T1 with the human plasminogen activation system. Therefore, loss of SpeB activity in a sub-population of *S. pyogenes* M1T1 at the site of infection results in accumulation of surface plasmin activity thus triggering systemic spread.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

ABBREVIATIONS

ADP	adenosine diphosphate
ANGIS	Australian National Genomic Information Service
Ap	ampicillin
APAF	Australian Proteome Analysis Facility
APC	antigen presenting cell
APS	ammonium persulfate
APSGN	acute post-streptococcal glomerulonephritis
ARF	acute rhematic fever
ATP	adenosine triphosphate
bp	base pairs
BSA	bovine serum albumin
°C	degrees Celsius
C3	complement component 3
CHAPS	3-[3-(cholamidopropyl)-dimethyl-ammonio]-1-propane sulfonate
cm	centimetre
Cpa	collagen-binding protein
CRS	closely related to SIC
C-terminal	carboxy-terminal
DAB	3,3'-diaminobenzidine
dH_2O	sterile glass distilled water
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DRS	distantly related to SIC
DTT	dithiothreitol
E. coli	*Escherichia coli*
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme linked immunosorbent assay
ExPASy	expert protein analysis system
FAFLP	fluorescent amplified fragment length polymorphism
FbaA	fibronectin-binding protein A

FbaB	fibronectin-binding protein B
FBP54	fibronectin-binding protein 54
g	acceleration due to gravity 9.8 ms^{-2}
g	gram
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAS	group A *Streptococcus*
GBS	group B *Streptococcus*
GRAB	G-related α_2-macroglobulin-binding protein
GTP	guanosine triphosphate
h	hour
HCl	hydrochloric acid
HDL	high density lipoprotein
HRG	histidine-rich glycoprotein
HRP	horseradish peroxidase
HtrA	high temperature requirement protease A
H_2O_2	hydrogen peroxide
IdeS	IgG-degrading enzyme of *Streptococcus pyogenes*
IEF	isoelectric focusing
Ig	immunoglobulin
IPG	immobilised pH gradient
IPTG	isopropyl-β-D-thiogalactoside
kb	kilo bases
kDa	kilo-Dalton
Km	kanamycin
kV	kilo volts
L	litre
LB	Luria Bertani
LTA	lipoteichoic acid
M	molar
m	milli
mA	milliamps
MAC	membrane attack complex
MALDI-TOF	matrix-assisted laser desorption ionisation time-of-flight
MHC	major histocompatibility complex
min	minutes

MLEE	multilocus enzyme electrophoresis
Mlp	M-like proteins
MLST	multilocus sequence typing
MOPS	3-(N-morpholino)propanesulfonic acid
Mrp	M-related proteins
MS	mass spectrometry
MSCRAMM	microbial surface component recognising adhesive matrix molecule
MWCO	molecular weight cut off
µ	micro
n	nano
N-terminal	amino terminal
NADP	nicotinamide adenine dinucleotide phosphate
NADP-GAPDH	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase
NCBI	National Centre for Biotechnology Information
Ni-NTA	nickel-nitrilotriacetic acid
nm	nanometres
NPBP	nephritis plasmin-binding protein
NT	Northern Territory
OD	optical density
Ω	ohm
PAGE	polyacrylamide gel electrophoresis
PAI-1	plasminogen activator inhibitor type 1
PAI-2	plasminogen activator inhibitor type 2
PAM	plasminogen-binding group A streptococcal M-like protein
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFBP	*S. pyogenes* fibronectin-binding protein
pI	isoelectric point
Plr	plasmin receptor
PMN	polymorphonuclear leukocytes
pmol	pico moles
PMSF	phenylmethylsulfonyl fluoride
PrtF2	protein F2
PVDF	polyvinylidene difluoride
r	resistant

®	registered
RFLP	restriction fragment length polymorphism
RGD	arginine-glycine-aspartic acid
RHD	rheumatic heart disease
RNA	ribonucleic acid
RNase A	ribonuclease A
rpm	revolutions per minute
s	sensitive
SA	streptavidin
S. agalactiae	*Streptococcus agalactiae*
SAGP	streptococcal acid glycoprotein
S. aurues	*Staphylococcus aureus*
SclA/SclB	streptococcal collagen-like protein
SCPA	streptococcal C5a peptidase
SDH	streptococcal surface dehydrogenase
SDS	sodium dodecyl sulfate
sec	seconds
SEN	streptococcal surface enolase
SfbI	streptococcal fibronectin-binding protein I
SfbII	streptococcal fibronectin-binding protein II
SfbX	streptococcal fibronectin-binding protein X
SibA	secreted immunoglobulin-binding protein from GAS
SIC	streptococcal inhibitor of complement
ska	streptokinase gene
SLS	streptolysin S
SLO	streptolysin O
SMEZ	streptococcal mitogenic exotoxin Z
SOF	serum opacity factor
Spe	streptococcal pyrogenic exotoxin
S. pneumoniae	*Streptococcus pneumoniae*
S. pyogenes	*Streptococcus pyogenes*
SSA	streptococcal superantigen
SSS	standard sample solubilisation solution
STSS	streptococcal toxic shock syndrome
TCA	trichloroacetic acid

TEMED	N,N,N',N'-Tetramethylethylenediamine
TF	trigger factor
THBY	Todd Hewitt broth, yeast supplemented
™	trade mark
tPA	tissue-type plasminogen activator
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
Triton® X-100	t-octylphenoxypolyethoxyethanol
2D	two-dimensional
uPA	urokinase-type plasminogen activator
UV	ultraviolet
V	volt
v/v	volume/volume
Vir	virulence type
W	watts
wt	wild-type
w/v	weight/volume
X	times
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-thiogalactopyranoside

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