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Is plasminogen deployed as a virulence factor by Northern Territory group A streptococcal isolates during invasive disease?

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Is plasminogen deployed as a virulence factor by Northern Territory group A streptococcal isolates during invasive disease?

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Certification

I, Fiona C. McKay, declare that this thesis, submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy in the School of Biological Sciences, University of Wollongong, is entirely my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Fiona C. McKay

22 December 2005

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Sanderson-Smith, M.L., McKay, F.C., Ranson, M. and Walker, M.J. (2004) Subversion of the plasminogen activation system by *Streptococcus pyogenes*: mounting evidence to implicate the human protease plasmin in disease processes. Current Trends in Microbiology 1: 75-85.

McKay, F. C., McArthur, J. D., Sanderson-Smith, M. L., Gardam, S., Currie, B. J., Sriprakash, K. S., Fagan, P. K., Towers, R. J., Batzloff, M. R., Chhatwal, G. S., Ranson, M. and Walker, M. J. (2004). Plasminogen binding by group A streptococcal isolates from a region of hyperendemicity for streptococcal skin infection and a high incidence of invasive infection, Infection and Immunity, 72, 364-70.

Walker, M.J., McArthur, J.D., McKay, F.C, and Ranson, M. (2005) Is plasminogen deployed as a *Streptococcus pyogenes* virulence factor? Trends in Microbiology, 13:308-13.

Cole, J.N., McArthur, J.D., McKay, F.C., Sanderson-Smith, M.L., Cork, A.J., Ranson, M., Rohde, M., Itzek, A., Sun, H., Ginsburg,D., Kotb, M., Nizet, V., Chhatwal, G.S. and Walker, M.J. Trigger for group A streptococcal M1T1 invasive disease, FASEB Journal *(accepted 31 March 2006).*

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List of Abbreviations

A_{600}, A_{405}	absorbance measured at 600 or 405 nm respectively
ANOVA	analysis of variance
APSGN	acute post-streptococcal glomerulonephritis
bp	base pairs
°C	degrees Celcius
CD	cluster of differentiation
cfu	colony-forming units
cpm	counts per min
C-terminal	carboxy-terminal
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E64	trans-epoxysuccinyl-l-leucylamido-(4-guanidino)butane
εACA	ε-aminocaproic acid
ECM	extracellular matrix
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
Fbp54	fibronectin-binding protein 54
Fg	fibrinogen
Fg-R	fibrinogen receptor
FSD	fibrinogen-streptokinase dependent
g	units of force
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAS	group A streptococcus
Glu-plasminogen	native plasminogen bearing an amino-terminal glutamine residue

h	hour
HLA	human leukocyte antigen
IgG	immunoglobulin G
Inv	invasive
K1-5	kringles 1-5
kb	kilobases
kDa	kilodaltons
KLH	keyhole limpet hemocyanin
l	litres
Lys-plasminogen	plasminogen bearing an amino-terminal lysine residue
m	milli
µ	micro
Mga	multiple gene regulator in group A streptococci
min	minutes
M	molar
m	metres
n	nano
NPBP	nephritis plasmin binding protein
N-terminal	amino-terminal
NT	Northern Territory (of Australia)
NTP	N-terminal peptide
PAI-1/-2	plasminogen activator-inhibitor type 1/2
PAM	plasminogen-binding group A streptococcal M-like protein
PBS	phosphate-buffered saline
PCR	polymerase chain reaction

PD	protease domain
PINT	phosphate isotonic sodium buffer containing Tween
Plg	plasminogen
Plr	plasmin receptor
Plg-R	plasminogen receptor
PMSF	phenylmethylsulfonyl fluoride
PPACK	D-Phe-Pro-Arg-chloromethylketone dihydrochloride
protein GRAB	protein G-related α_2-macroglobulin-binding protein
PVDF	polyvinyl difluoride
RT	room temperature
s	seconds
SCID	severe combined immunodeficient
SD	standard deviation
SDH	streptococcal dehydrogenase
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	standard error of the mean
SEN	streptococcal surface enolase
ska	streptokinase gene
SpeB	streptococcal pyrogenic exotoxin
STSS	streptococcal toxic shock syndrome
TAE	Tris acetate-EDTA
TBS(T)	Tris-buffered saline (containing Tween)
TE	Tris-EDTA
TEA	tranexamic acid
THY	Todd-Hewitt broth containing 1% yeast

tPA	tissue plasminogen activator
uncomp	uncomplicated infection
uPA	urokinase plasminogen activator
uPAR	urokinase plasminogen activator receptor
UV	ultraviolet
V	volts
vir	virulence (regulon)
Vmax	maximum reaction velocity
wt	wildtype
w/v	weight per volume

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Abstract

Group A streptococcal (*S. pyogenes*; GAS) infection is endemic in the Northern Territory of Australia, and the rates of invasive GAS disease and post-infection sequelae are among the highest reported in the world. Plasminogen is a potent human protease sequestered to the GAS cell-surface by plasminogen and fibrinogen receptors and activated by GAS streptokinase and host plasminogen activators. The critical role of plasminogen in GAS invasion was recently demonstrated in a human plasminogen transgenic mouse model of infection.

The aim of this study was to determine whether plasminogen is deployed as a virulence factor in invasive GAS disease, with particular reference to the Northern Territory of Australia. This question was first approached from an epidemiological perspective, comparing Northern Territory GAS isolates from invasive infections with those from uncomplicated infections for their interaction with the plasminogen system. Plasminogen binding; plasminogen receptor expression and genetic variation; fibrinogen binding; streptokinase expression, activity and genetic variation; streptococcal pyrogenic exotoxin B (SpeB) expression and activity; and acquisition of cell-surface plasmin in human plasma were characterised for 29 GAS isolates of known clinical origin from the Northern Territory. The second approach to determining the role of plasminogen in invasive disease in the Northern Territory was to investigate GAS infection using a human plasminogen transgenic mouse model. A subset of Northern Territory GAS isolates selected for different *in vitro* plasminogen activation characteristics was tested for virulence in this model.

This study revealed that Northern Territory GAS isolates from invasive disease cases acquire more plasminogen than isolates from uncomplicated infections, however they do not produce more streptokinase nor acquire more cell-surface plasmin after incubation in human plasma *in vitro*. Presence of the gene for the plasminogen-binding group A streptococcal M-like protein (PAM) conferred upon GAS isolates a strikingly different profile of interaction with plasminogen, characterised by higher plasminogen binding and plasmin acquisition in plasma. Differences in the catalytic specificity of streptokinase secreted by *pam*-positive and *pam*-negative isolates were identified and their allelic determinants investigated. A new model of GAS cell-surface plasminogen activation is proposed for *pam*-positive isolates. To characterise the role of streptokinase and the cysteine protease SpeB in acquisition of cell-surface plasmin activity in human plasma, such acquisition was compared in GAS deletion mutants for genes encoding these proteins and the corresponding wildtype strains. The dramatic reduction of GAS cell-surface plasmin activity by SpeB may significantly disable GAS invasive potential. Infection studies in the human plasminogen transgenic mouse model revealed the critical role of plasminogen in virulence of some Northern Territory isolates, however *in vitro* plasminogen activation characteristics do not predict clinical phenotype nor virulence in the transgenic mouse model. The results suggest an important and complex interaction between plasminogen and other host and/or bacterial factors in establishing invasive GAS infection in the Northern Territory of Australia.