Initiation of invasive disease in M1T1 group A streptococcus

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Initiation of Invasive Disease in M1T1
Group A Streptococcus

A Thesis Submitted in Fulfilment of the Requirements
For the Award of the Degree

Doctor of Philosophy (PhD)

From the

University of Wollongong

By

Andrew Hollands

School of Biological Sciences

2009
DECLARATION

I, Andrew Hollands, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy (PhD), in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Andrew Hollands

30 November, 2009
ABSTRACT

*Streptococcus pyogenes* (group A streptococcus; GAS) is an important human pathogen that colonizes epithelial and mucosal surfaces. Group A streptococcal disease can be relatively minor, such as streptococcal pharyngitis, or severe and life-threatening, such as necrotizing fasciitis. There has been a resurgence of severe infection with GAS since the mid-1980s that has been paralleled by the emergence of a globally disseminated clone, M1T1. The M1T1 clone of GAS presents as the most common cause of streptococcal pharyngitis in developed countries and are also overrepresented in cases of severe infection.

Most invasive bacterial infections are caused by species that more commonly colonize the human host with minimal or no symptoms. Although phenotypic or genetic correlates underlying a bacterium’s shift to enhanced virulence potential have been studied, the *in vivo* selection pressures governing such shifts are poorly understood. The globally disseminated M1T1 clone of GAS is linked with the rare but life-threatening syndromes of necrotizing fasciitis and toxic shock syndrome. Mutations in the group A streptococcal control of virulence regulatory sensor kinase *(covR/S)* operon are associated with severe invasive disease, abolishing expression of a broad spectrum cysteine protease (SpeB) and allowing the recruitment and activation of host plasminogen on the bacterial surface. This study describes how a bacteriophage-encoded group A streptococcal DNase (Sda1), which facilitates the pathogen’s escape from neutrophil extracellular traps (NETs), can serve as a selective force for *covR/S* mutation. The results provide a paradigm whereby horizontal gene transfer and natural selection exerted by the innate immune system
generate hypervirulent bacterial variants with increased risk of systemic dissemination.

This study sought to investigate if there was a cost of fitness associated with \textit{covR/S} mutation that counterbalances the dramatic increase in virulence. It was found that \textit{covR/S} mutant bacteria had reduced capacity to bind fibronectin and collagen, both components of the extracellular matrix bound by streptococcal adhesins. The \textit{covR/S} mutant strain examined in this study also showed reduced capacity to bind to epithelial cell layers as a consequence of increased capsule expression. This mutant strain displayed reduced capacity to form biofilms. An animal model of skin colonization was used to show that the \textit{covR/S} mutant strain has a colonization defect. This reduced capacity to colonize presents an explanation as to why hypervirulent \textit{covR/S} mutant M1T1 group A streptococci are not rapidly spread amongst the community.

The role of SpeB in the course of infection is still unclear. This study utilized a SpeB-negative M1T1 clinical isolate, 5628, with a naturally occurring mutation in the gene encoding the regulator RopB, to elucidate the role of RopB and SpeB in systemic virulence. Allelic exchange mutagenesis was used to replace the mutated \textit{ropB} allele in 5628 with the intact allele from the well characterized isolate 5448. The inverse allelic exchange was also performed to replace the intact \textit{ropB} in 5448 with the mutated allele from 5628. An intact \textit{ropB} was found to be essential for SpeB expression. While the \textit{ropB} mutation was shown to have no effect on haemolysis of RBCs, extracellular DNase activity or survival in the presence of neutrophils, strains with the mutated \textit{ropB} allele were less virulent in murine systemic models of
infection. An isogenic SpeB knockout strain containing an intact RopB showed similarly reduced virulence. Microarray analysis found genes of the SpeB operon to be the primary target of RopB regulation. These data show that an intact RopB and efficient SpeB production are necessary for systemic infection with GAS.
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First and foremost I would like to thank my supervisors, Prof. Mark Walker, and Prof. Victor Nizet, for their support and guidance throughout my PhD candidature. I would also like to thank the members of the Walker lab and the Nizet lab for their friendship, support, guidance and encouragement over the last few years.

I extend my thanks to all contributors and co-authors of the work contained in this thesis: Ramy Aziz, John Buchanan, Gursharan S. Chhatwal, Jason Cole, Katrin Dinkla, Anna Henningham, Rita Kansal, Josh Kirk, Malak Kotb, Jason McArthur, Sarah R Osvath, Morgan Pence, Martina Sanderson-Smith, Amelia Simpson, Anjuli Timmer, Lynne Turnbull and Cynthia B Whitchurch. I would also like to thank the following for their assistance: Arthur Jeng and Kalpana Chalasani for constructing the isogenic mutant 5448Δsmez; Ramy Attia for assisting with real-time PCR; Grant Ellmers and Robert Dinnervill for illustrating Figure 2.5; Anna Cogen for her assistance with a murine model of colonization; William L. Taylor (University of Tennessee, Molecular Resource Center) for his help and guidance in hybridizing and scanning microarrays.

Last but definitely not least, I wish to express my deep gratitude to my family and my wife Terrie for their understanding and encouragement that has helped me maintain dedication throughout the course of my PhD.
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ABBREVIATIONS

°C  degrees Celsius
aa  amino acid
Ab  antibody
ANOVA analysis of variance
APSGN acute post-streptococcal glomerulonephritis
ARF  acute rheumatic fever
BLAST  basic local alignment search tool
bp  base pair
CCD  charge-coupled device
cDNA complementary DNA
CFU  colony forming units
Cm  chloramphenicol
Co  collagen
CovR/S control of virulence regulator/sensor
DNA  deoxyribonucleic acid
DTT  dithiothreitol
E. coli  *Escherichia coli*
ECM  extracellular matrix
EDTA  ethylenediaminotetraacetic acid
Erm  erythromycin
FBP  fibronectin binding protein
FCT  fibronectin-binding, collagen-binding, T-antigen
Fn  fibronectin
$g$  acceleration due to gravity (9.8 m s$^{-2}$)
GAPDH  glyceraldehyde-3-phosphate dehydrogenase
GAS  group A streptococcus
GEO  Gene Expression Omnibus
h  hours
HRP  horseradish peroxidase
IgA  immunoglobulin A
IgG  immunoglobulin G
kDa  kilodaltons
LA  Luria-Bertani agar
LB  Luria-Bertani broth
LTA  lipoteichoic acid
M  molar
MBC  minimum bactericidal concentration
MF  mitogenic factor
MHC  major histocompatibility complex
MIAME minimum information about a microarray experiment
MIC  minimum inhibitory concentration
min  minutes
ml  millilitres
mM  millimolar
mm  millimetres
Mrp  M-related protein
<table>
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<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
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<tr>
<td>NET</td>
<td>neutrophil extracellular trap</td>
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<td>ng</td>
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<td>nt</td>
<td>nucleotide</td>
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<td>OD</td>
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<td>ORF</td>
<td>open reading frame</td>
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<td>PAI</td>
<td>plasminogen activator inhibitor</td>
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<td>serum opacity factor</td>
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<td><em>Streptococcus pyogenes</em> cell envelope protease</td>
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<td>Todd-Hewitt broth</td>
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<td>TNF</td>
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