

Project Details	
Project Code	MRC23IIARBr Nobbs
Title	Sticky but not sweet: deciphering how Streptococcus bacteria drive clot formation and heart disease
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	Streptococcus bacteria cause heart disease infective endocarditis (IE), for which novel therapies are urgently needed. Bacterial protein PadA is critical to IE pathogenesis but how it functions is unknown. This project combines molecular/clinical microbiology, host immunity and structural biology to identify the mechanisms by which PadA aids bacterial survival in blood and drives the harmful clot formation seen with IE. Such insight is critical to IE therapy design.
Description	<p>Heart disease is the leading cause of death in the developed world. Viridans group Streptococcus bacteria (VGS) are a major cause of infective endocarditis (IE), a severe form of heart disease characterised by clot formation on heart valves. IE has a high mortality rate, is lethal if untreated, and treatment often fails due to poor antibiotic penetration into infective clots or antibiotic resistance. This indicates an unmet clinical need for development of non-antibiotic-based strategies for the management of IE. This project aims to identify the precise molecular mechanisms by which VGS can survive within the bloodstream and drive the unwanted clot formation associated with IE. Such mechanistic understanding is a critical first step to the development of novel IE therapies. The association of VGS with IE derives from their capacity to bind and activate platelets on heart valves. Our work with VGS <i>Streptococcus gordonii</i> has shown that surface protein PadA is critical to IE pathogenesis. PadA triggers platelet activation via receptor GPIIb/IIIa, modulates neutrophil extracellular trap (NET) formation, promotes bacterial survival in blood, and contributes to virulence in a rabbit IE model. However, how PadA mediates these effects at the molecular level is unknown. Furthermore, while studies have focused on <i>S. gordonii</i>, homologues of PadA are found across several VGS species but it is not known if these confer similar functional capabilities. This project will address these critical questions via the following objectives:</p> <p>1: ID the mechanisms by which PadA modulates platelet/neutrophil behaviour Using a panel of bacterial mutants, recombinant protein (rPadA) and primary human cells, studies will reveal the complex cascade of downstream signalling events mediated by PadA that culminate in platelet spreading and aggregation to drive clot formation. Neutrophil activation and NET formation will be monitored to assess if platelets and/or VGS trigger the release of NETs to serve as a scaffold to enhance bacteria-platelet clot formation.</p> <p>2: ID the mechanisms by which PadA promotes survival in blood Bacterial mutants and/or rPadA will be used to investigate the capacity for PadA to evade primary human neutrophil- or complement-mediated killing. Studies will explore phagocytic killing, cytokine release, serum bactericidal activity, binding of complement inhibitors/serum proteins and deposition of complement factors.</p> <p>3: Structural characterisation of PadA Structural resolution of PadA is critical for the design of targeted therapeutic strategies. Crystallisation and co-crystallisation with known ligands (e.g. GPIIb/IIIa) will be performed with domains of rPadA. Stability and flexibility will be</p>

	<p>assessed with a combination of biophysical and hydrodynamic approaches to understand how PadA functions under the shear forces of the cardiovascular system. 4: Assessment of PadA homologues from clinical isolates Severn Pathology has a well-curated collection of VGS isolated from the blood of IE patients. This will be exploited to extend PadA studies beyond <i>S. gordonii</i>, enhancing the clinical relevance of this work. IE strains will be screened for PadA (gene/protein) and compared with VGS isolates from non-IE patients. Functional capabilities of PadA homologues with regards to platelet/neutrophil interactions and blood survival will be determined. The student and supervisory team will have regular meetings at which next steps for project development will be discussed. It is expected that the balance of these sessions will shift from supervisor to student during the project life cycle as the student builds skills and confidence as an independent researcher.</p>
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