Pore formation and regulation of recently identified *Bacteroides fragilis* Cholesterol-dependent Cytolysin Like (CDCL) proteins.

Riya Joseph [1,2], Michelle P. Christie [1,2], Bronte A. Johnstone [1], Michael Gorman[1], Craig J. Morton [1,3], Rodney K. Tweten [4], Michael W. Parker [1,2,5]

[1] Department of Biochemistry and Pharmacology, Bio21 Institute, University of Melbourne, Parkville, Victoria, Australia, [2] ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Bio21 Institute, University of Melbourne, Parkville, Victoria, Australia, [3] CSIRO Biomedical Manufacturing Program, Clayton, Victoria, Australia, [4] Department of Microbiology and Immunology, University of Oklahoma, Oklahoma City, Oklahoma, USA. [5] Australian Cancer Research Foundation Rational Drug Discovery Centre, St Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia.

Email: riyaj@student.unimelb.edu.au

Bacteroides fragilis are gram-negative anaerobic bacteria that are found in the human gut. Although generally commensal, they can become pathogenic under conditions of impaired intestinal barrier function, leading to various diseases. B. fragilis bacteria are shown to produce a novel class of pore forming proteins termed as Cholesterol Dependent Cytolysin Like proteins (CDCLs) (1). These pore-forming proteins produced by B. fragilis play a crucial role in their survival within the intestinal environment by helping them target competing microorganisms. Although pore forming proteins have been studied for the past four decades, we still don't clearly understand the mechanism of action of these proteins, especially how they are inserted into target membranes (2).

We are currently investigating the structure and pore-forming activity of two CDCL proteins (Bf long and Bf short) from B. fragilis. These proteins when proteolytically activated at their target membrane, forms a two-component beta-barrel pore comprising of approximately 30 monomers. Interestingly, B. fragilis also produces a surface lipoprotein named Bacteroides CDCL immunity protein (BcdI) that protects it from its own toxins (3).

We employ a diverse array of structural biology techniques, including X-ray crystallography to determine monomer structures, small-angle X-ray scattering (SAXS) for solution-state analysis, and electron microscopy to investigate the pore complex of these proteins. Progress towards these aims, including crystal structures of Bf short and BcdI, solution structures and EM images of BcdI and CDCLs will be discussed. These findings, along with our ongoing research, contribute to our understanding of the pore formation mechanism and regulation of Bf CDCLs, shedding light on how B. fragilis utilizes these virulence factors to its advantage.

References:

1. Evans JC, Johnstone BA, Lawrence SL, Morton CJ, Christie MP, Parker MW, et al. A Key Motif in the Cholesterol-Dependent Cytolysins Reveals a Large Family of Related Proteins. mBio. 2020;11(5).
2. Johnstone BA, Joseph R, Christie MP, Morton CJ, McGuiness C, Walsh JC, et al. Cholesterol-dependent cytolysins: The outstanding questions. IUBMB Life. 2022.
3. Abrahamsen HL, Sanford TC, Collamore CE, et al. Distant relatives of a eukaryotic cell-specific toxin family evolved a complement-like mechanism to kill bacteria. *Nat Commun*. 2024;15(1):5028. Published 2024 Jun 12. doi:10.1038/s41467-024-49103-5