Project Details	
Project Code	IIAR27Br Laabei
Title	Developing targeted covalent macrocycles to combat immune-mediated diseases
Research Theme	Infection, Immunity & Antimicrobial Resistance
Project Type	Wet lab
Summary	Complement is an ancient, conserved system vital for defence against pathogens and maintaining tissue homeostasis. However, dysregulation can cause damage contributing to many autoimmune and inflammatory diseases. Despite its therapeutic potential, few complement-targeting drugs have been approved for clinical use. Selective inhibition of key complement proteins offers a promising approach. This proposal aims to develop targeted covalent macrocycle inhibitors of complement serine proteases using molecular, chemical, and structural biology tools, advancing next generation complement therapies toward clinical application.
Description	Background: Complement is an evolutionary conserved system, essential for detecting pathogens and maintaining tissue homeostasis. The complement system is composed of soluble or surface-expressed proteins and can be activated by three different routes, the classical, lectin or alternative pathway, each with distinct initiating mechanisms. All three pathways converge at the level of the central C3 protein, which after proteolytic cleavage is responsible for mediating phagocytosis of foreign bodies by phagocytes. C3 proteins also initiate the proteolytic cleavage of C5, enabling formation of the membrane attack complex (MAC), which results in lysis of susceptible cells. Dysregulation of complement activation can trigger a harmful cycle of inflammatory damage, inducing or aggravating a broad range of inflammatory and autoimmune diseases. While the clinical potential of targeting the complement system has long been recognized, the approval rate for complement-based drugs remains low. Precise inhibition of critical complement proteins that play a role in initiating and propagating complement activation is viewed as an exciting method of therapy and forms the basis of this PhD project. Aims and Overview: Complement-directed therapeutic development has disproportionately targeted proteins involved in C5 cleavage and activation, but this has a major drawback as broad-spectrum complement pathway inhibition increases patient susceptibility to bacterial infections. Several disease conditions are driven by pathway-specific complement activation. Consequently, development of pathway-specific inhibitors represents a promising therapeutic approach, which could allow for disease treatment without significantly increasing pathogen susceptibility. The aim of this PhD is to develop pathway-specific inhibitors by targeting serine proteases that participate in either the classical or alternative activation pathway. Specifically, we will target C1s, which is a serine protease that facilitates C3 activation via the classical pathway. Additionally, we will target factor D, which is a serine protease and rate limiting enzyme essential for the activity of C3 and C5 via the alternative pathway. Inhibitors of these enzymes could be used to treat diseases such as heparin-induced thrombocytopenia and atypical haemolytic uremic syndrome.

	Obj 1: Targeting C1s and Factor D Using Covalent Phage Display – Using a phage display screening platform developed in the Lovell lab, targeted covalent macrocycle (TCM) inhibitors will be identified for C1s and factor D. TCMs combine the properties of a macrocyclic peptide and an irreversible inhibitor and are particularly suited to engaging individual proteases. Indeed, the Lovell lab have identified highly selective TCM inhibitors for challenging viral, bacterial and cancer protease targets. Chemical linchpins containing serine-targeting electrophilic 'warheads' will be used to cyclise peptide-displaying phage libraries to generate billions of TCMs for screening against immobilised C1s and factor D. After multiple rounds of panning and amplification, next generation sequencing will be performed to identify TCMs that are selectively enriched against one of the protease targets. These TCMs will then be synthesized using solid-phase peptide synthesis and tested in objective 2. Obj 2: Characterisation of TCM inhibitors - The student will determine the anti-complement activity of enriched TCMs using biophysical techniques and complement-specific assays that will report on inhibition of specific protease targets. Dedicated C1s and FD protease activity assays will be performed using chromogenic substrates appropriate to each protease to assess TCM potency and selectivity (against a panel of structurally similar proteases). Chemical proteomics will be used to assess proteome-wide selectivity of hit TCMs in human blood. Optimised classical pathway or alternative pathway ELISA-based complement activation assays and sheep blood haemolytic assays will be used to examine complement inhibition. Obj 3: Structural basis of TCM mediated inhibition - The student will obtain co-crystal structures of hit TCMs with C1s and Factor D, revealing critical residue interactions and enabling structure-guided optimization of key molecule parameters such as selectivity and proteolytic stability. Outcome: TCMs for C1s and Factor D will be delivered with validated potency, selectivity and stability, forming the basis of a future MRC grant proposal to push novel complement-targeting molecules toward the clinic to address multiple autoimmune disorders. The student will receive training in chemistry, microbiology and molecular biology, which will position them strongly to succeed in a future academic or industrial research career. Opportunities for student ownership and steering: This is a highly interdisciplinary project with several opportunities for the student to steer the project based on their interests. This could include, but is not limited to, the following: (A) Focusing on phage display technology development by inventing novel chemical linchpins to allow diverse TCM libraries to be generated. (B) Focusing on structural biology by gaining expertise in X-ray crystallography/cryo-EM during TCM hit-to-lead optimisation. (C) Developing new bioinformatics pipelines to allow in-depth analysis of deep sequencing data from phage display screens. (D) Developing new complement activity assays that more closely resemble signalling in vivo. (E) Understanding the impact of selective complement inhibition on in vitro killing of encapsulated bacterial pathogens using naïve and immune serum (vaccinated individuals)

Can be completed part time?	No
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