Project Details	
Project Code	MRCIIAR24Ca Heurich
Title	Unravelling the molecular link between coagulation and immunity
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	The blood-based coagulation and immune complement systems are activated in response to infection or injury. These systems are generally viewed as distinct, but several protein interactions between these systems affect their function and may contribute to disease. We will use biochemical and biophysical methods such as SPR/Biacore and X-ray crystallography to characterize the first supra-molecular complex integral to blood coagulation and immune system crosstalk.
Description	Background: The blood clotting and innate immune/complement systems are both blood-based proteolytic cascades that are activated by pathway-specific triggers in response to infection or tissue injury. These systems are generally viewed as distinct, but have common evolutionary origins, and several interactions between these homologous systems have been reported. This complement and coagulation crosstalk can affect activation, amplification and regulatory functions in both systems and their dysregulation may contribute to disease. Initial studies performed by the Heurich lab in Cardiff has shown that thrombin, the blood clotting factor responsible for generating the fibrin clot, can enzymatically cleaves and interact with several complement proteins, including complement C3, and factor H, affecting both complement and coagulation functions [1,2]. Within the complement cascade, the cleavage of C3 is normally catalysed by the complement C3 convertase, yielding activated fragments anaphylatoxin C3a and opsonin C3b. C3b is then further cleaved into iC3b by complement factors I and H. C3a, C3b and iC3b have functions in immune cell recruitment and act as opsonins in the interaction with phagocytosing immune cells. Thrombin can also bind and cleave C3 into C3a and C3b, and subsequently into iC3b. Thrombin also binds factor H, which affects thrombins' catalytic ability. These molecular crosstalk interactions may affect activity and amplification mechanisms in response to infection or tissue injury when these systems are often synergistically activated. While we have some insight into the structure of C3 with the C3 convertase, the detailed binding interactions, affinity, binding sites and the molecular complex of the thrombin interaction with complement proteins remain unresolved. Further, the comparable activity and function of the thrombin-generated cleavage products in their ability to promote inflammation and phagocytosis remain unclear. Aims: This PhD project seeks to gain understanding of the detailed molecular aspects of the thrombin interaction with C3, and to elucidate the differences compared with the C3 convertase at a molecular level. Heurich has specific experience in analysing the structure-function relationship of complement and coagulation proteins, including C3 and thrombin [3-6], and van den Elsen has long-standing expertise in structural analyses, including complexes of complement components [8-12]. Objective 1) involves the study of individual complement proteins C3, C3b, iC3b and their interactions with thrombin using biophysical methods (SPR-Biacore,ITC). Objective 2) Next, we will perform functional studies utilizing established methods [8] to compare thrombin-generated C3b, iC3b and C3a, in their ability to

	bind to their receptors (CR1, CR3) and their ability to promote phagocytosis (Obj 1&2, Heurich, Cardiff). Objective 3) Initial studies performed using low-resolution structural techniques such as small-angle X-ray scattering (SAXS) will provide a first glimpse of the C3-thrombin molecular complex in solution. Finally, Objective 4) includes X-ray crystallographic analyses [9-11] of the C3-thrombin complex to help elucidate the atomic details of the interaction surfaces, enabling further molecular dynamics and molecular docking studies. [12-16] (Obj 3&4: van den Elsen, Bath; Mikolajek, Diamond Synchroton,Harwell). Subsequent mutational studies at the protein interfaces involved in the complex will provide information about potential ways to interfere with complex formation. The detailed mechanistic insights gained from this study will help to inform the development of targeted therapies for the treatment of inflammatory diseases resulting from complement and coagulation crosstalk dysregulation.
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