Probing the molecular activation of complement component C5 using a novel antibody toolbox

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Project description:

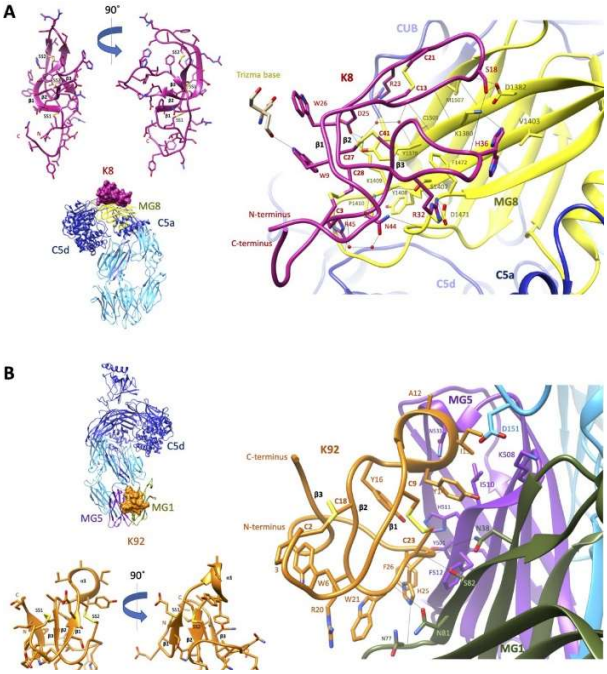

Figure 2. Co-crystal structures of C5 with knob domain peptide modulators revealing the mechanistic basis for allosteric inhibition of C5 (Macpherson et al, ELife, 2021).

Background: The complement system is a central component of innate immunity, a network of plasma proteins, which provides defence against infection and efficient removal of dead cells. Because of its complexity and destructive character, complement is tightly regulated by an array of fluid-phase and membrane regulators. Activation of complement is triggered in several ways, leading to the formation of enzymes, termed convertases, that cleave the central component C3 to C3a and C3b. C3b forms the nidus for formation of the C5 convertase, which in turn cleaves C5, the principal effector of the terminal portion of the complement cascade and subject of this proposal, into its fragments C5a and C5b. Once cleaved, the C5a fragment acts as a potent chemoattractant recruiting immune cells to sites of complement activation, whilst C5b contributes to the membrane attack complex (MAC) a molecular assembly involved in the efficient killing of microbial pathogens.

Aims: This project aims to gain a detailed molecular understanding of the aspects that control the cleavage of C5 by the C5 convertase enzyme complexes. For this purpose, we have developed a unique molecular toolkit comprised of a recombinant Llama anti-C5 VHH library (nanobodies) and a novel class of low molecular weight antibody fragments derived from cow antibodies, known as knob domain peptides, that have been developed by UCB-Pharma in collaboration with the University of Bath. This tool kit, in combination with our libraries of recombinant anti-C5 Fabs and known parasite-derived inhibitors of C5, will be available to the PhD student 1) to profile for C5 binders (Bath/UCB). Subsequently, 2) functional studies will be employed to assess inhibitory and non-inhibitory binders (UCB/Cardiff) and assign a putative mode of action. Finally, the most promising binders will subjected to 3) structural analyses using X-ray crystallography (Bath) and computational methods.

The student will gain experience in a wide range of techniques across different disciplines and Universities, including protein biochemistry and X-ray crystallography (Bath/UCB), complement activity analyses (Cardiff) and computational techniques, particularly molecular dynamics simulation (UCB). We intend to use our molecular probes to gain a detailed mechanistic insight into the activation of C5, informing the development of targeted therapies for the treatment of inflammatory diseases resulting from complement dysregulation, including the acute respiratory distress syndrome linked to COVID-19. Results will be communicated in publications, and at conferences. Outcomes from the project will have immediate impact on the many academic and commercial groups working on complement therapies.