Project Details	
Project Code	MRC23IIARCa Rhys
Title	De novo synthekines for treatment of autoimmune diseases
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	Inflammation is essential for responding to infection and tissue damage. Its dysregulation, however, causes autoimmune diseases and chronic inflammation. Modulating cytokine activity has emerged as an important therapy for autoimmune conditions such as rheumatoid arthritis and uveitis. In this project you will computationally design new-to-nature proteins that mimic cytokines and test their therapeutic potential in autoimmunity.
Description	Importance Cytokine blockers, cytokine receptor blockers and JAK inhibitors have emerged as frontline drugs that have significantly improved treatment in rheumatoid arthritis (RA) and uveitis, although not all patients respond. These therapies systemically suppress pro-inflammatory responses. An unexplored and complementary strategy would be to develop therapeutics that promote an anti-inflammatory response. Pre-clinical studies by us and others have highlighted the potential of regulatory cytokines such as IL-10 & IL-27. However, with exception of type I IFNs in multiple sclerosis, administration of cytokines has failed to show efficacy in clinical trials, which could be because of their short half-lives and failure to target the relevant tissue. In this project we will use computational protein design to overcome these pitfalls by designing a highly potent, highly stable, non-immunogenic IL-27 mimic that can be localised to sites of inflammation. Objectives 1. Computationally design sequences for bispecific synthekines (Rhys lab) The student will explore and learn about the very latest in neural-network-based computational algorithms, including MaSIF-seed and ProteinMPNN. They will then choose an algorithm for designing binders against IL-27Rα and gp130. The design process will produce hundreds of sequences, which we will pare down to the 30 top-scoring proteins (15 against IL-27Rα and 15 against gp130) ranked according to a set of metrics. Finally, genes encoding for the 30 candidates will be ordered. 2. Identify sequences with excellent biophysical properties (Rhys lab) The proteins will be produced by recombinant protein expression in E. coli. Initially they will test the expressibility and solubility of each protein. Designs that can be expressed in sufficient quantity will be purified via affinity tag and size-exclusion chromatography (SEC). They will test the proteins' propensity to oligomerise and aggregate using analytical SEC, and their solution-phase secondary structure and thermal stability using circular dichroism. 3. Develop high affinity binders for against IL-27Rα and gp130 (Rhys & Tomlinson labs) The binding affinity of promising designed proteins for their respective targets, i.e. IL-27Rα or gp130, will be measured by surface plasmon resonance. It is likely that the computational-designed binders will have weak (mM to microM) affinities to their targets. To increase affinity, the student will visit the Tomlinson lab, where they will conduct phage-display experiments to isolate variants with nM to fM binding affinity. The two highest affinity candidates against IL-27Rα and gp130 will be fused by a structured linker to give bispecific synthekines. Finally, peptide tags that target the human synovial microvascular endothelium

	or uvea will be fused to our bispecific synthekines. 4. Demonstrate functional bispecific synthekines in in vitro/in vivo assays (Jones & Dick labs) The function of the synthekines will first be tested using established in vitro CD4 T cell culture assays and compared to commercial recombinant IL-27. Here, the ability of the synthekines to activate intracellular signalling (phospho-STAT1 and STAT3) downstream of IL-27Rα and gp130 engagement will be determined by flow cytometry. The ability of synthekines to inhibit pathogenic IL-17-producing CD4 (Th17) cell development, a hallmark of IL-27 behaviour, will be tested using in vitro differentiation assays. Finally, the therapeutic potential of the best candidates will be tested using in vivo mouse models of RA (antigen-induced arthritis) and experimental autoimmune uveoretinitis. Consistent with our data demonstrating anti-inflammatory roles for IL-27 in these models, mice will be administered synthekines and disease severity tracked through clinical scoring involving histopathology (for arthritis) and ocular imaging (for uveitis) and the effect on joint- and ocular-infiltrating pathogenic CD4/Th17 cells determined by flow cytometry.
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