Project Details	
Project Code	MRCIIAR24Ca Fielding
Title	Understanding the interactions of Dengue virus with the immune system and its impact on disease
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Natural killer (NK) cells are critical for killing virus-infected cells but can also cause immunopathology. This project investigates whether Dengue virus manipulates NK ligands to prevent NK cell killing and whether NK cells contribute to dengue pathogenesis. This has important implications for understanding NK cells and immunotherapy development. The student will receive cutting-edge training in virology, immunology, single cell RNA sequencing and proteomics.
Description	Dengue virus (DENV) is a flavivirus with four distinct serotypes (DENV 1-4). It is spread by mosquito bites and is the most prevalent mosquito-borne viral disease of humans (390 million people per year). DENV infection may result in an asymptomatic infection, a febrile illness or a more severe life-threatening syndrome characterized by plasma leakage and bleeding. There are currently no dengue therapeutics and only a partially protective vaccine. Natural killer (NK) cells are critical components of the antiviral immune response. They are activated in response to the balance of activating and inhibitory ligands on the surface of the virus-infected cell. Infection alters this balance such that NK cells become activated releasing perforins and granzymes to lyse the target cell. As a result, viruses must antagonise this process to successfully establish infection. However, dysregulated NK cell activation can also cause tissue damage. Thus, NK cells can be both protective and detrimental in terms of disease outcome. Previous data suggests NK cells play an important role in Dengue virus pathogenesis (1). This project will investigate the interactions of the DENV-infected cells with NK cells and their impact on disease. This builds on previous work on CMV, SARS-CoV-2 and DENV (2-6). 1. Investigating the effect of DENV infection on NK cell activation. Replication-deficient DENV replicon expressing cells (BSL2) (5) will be used as target cells in a range of NK cell activation assays (CD107a degranulation, chromium release cytotoxicity assays, specific NK reporter cells) (2-4). Single cell RNA sequencing (scRNAseq) will be used to identify responding and non-responding NK cells. This aim will determine whether DENV infection alters NK cell function. 2. Identification of plasma membrane proteins modulated by DENV. NK activity is controlled by the levels of >25 different ligands on the target cell surface. Cell surface proteomics will identify those that are altered by infection, in an unbiased manner (as used in (2-4)). At defined time points post-infection, triplicate samples will be prepared for plasma membrane profiling (PMP) by biotinylating surface glycoproteins and streptavidin pulldown. Samples will be analysed and quantified in a labelled proteomics experiment. Proteins up-regulated or down-regulated >2.5 fold will be analysed for enriched pathways. As well as host ligands altered by infection, this will identify viral cell surface proteins potentially acting as direct immunomodulators and targets for antibody- dependent activation. Protein modulation will be confirmed by flow cytometry and analysis of glycoprotein maturation. This analysis will reveal whether Dengue modulates known NK ligands and may also

	identify novel viral/host proteins capable of affecting NK function. 3. Mapping of DENV-encoded NK cell modulators. Aims 1-2 will be followed up by identifying the viral open reading frames (ORFs) responsible for the effects seen. Where NK ligands are modulated, DENV ORFs will be expressed individually in cell lines and screened for their ability to modulate NK ligands and NK function. If an ORF modulates NK function but not a known NK ligands, PMP proteomics will identify novel candidate proteins. If the viral proteins are expressed on the cell surface, their capacity to hyperactivate NK cells via antibodies will be tested. Targeted experiments will be carried out using Dengue virus at BSL3. This aim will identify mechanisms that could be targeted to enhance NK recognition of DENV infection or those underlying unwanted NK activation and immunopathology. 1. Mathew Immunology 2018 doi:10.1111/imm.12928 2. Weekes et al. Cell 2014 doi: 10.1016/j.cell.2014.04.028, 3. Fielding et al. eLife 2017 doi: 10.7554/eLife.22206 4. Fielding et al. eLife 2022 doi: 10.7554/eLife.74489, 5. Naiyer et al. Science Immunol. 2017 doi: 10.1126/sciimmunol.aal5296 6. Zimmer et al. Nature Comm. 2019 doi:10.1038/s41467-019-11878-3
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