Project Details	
Project Code	MRCIIAR25Ca Fielding
Title	Systematic Characterisation of Inhibitory Ligands Encoded by Human Cytomegalovirus
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Human cytomegalovirus (HCMV) is the leading infectious cause of congenital birth defects and causes severe disease immunosuppressed individuals. Immune cells, such as T-cell and Natural killer (NK) cells, are important for the control of cytomegalovirus infection, but the virus employs many immune evasion strategies. Some target cellular inhibitory receptors to inhibit these immune cells. This project aims to increase our understanding of HCMV's interaction with the immune system and how it targets inhibitory receptors expressed by natural killer cells and T-cells to allow immune evasion suggesting future therapeutic avenues. The student will receive cutting-edge training in virology and immunology
Description	Immune cells are important for the control of cancers and viral infections. In particular, cytotoxic T-cell and Natural killer (NK) are able to sense healthy tissue and kill tumour and viral-infected cells. This process is controlled by cues they receive from proteins on their cell surface (receptors), that either provide activating or inhibitory signals telling them to kill or leave alone cells they encounter. A greater understanding of what these receptors recognise has led to therapies to activate immune cells for anti-cancer therapies e.g. recent checkpoint inhibitor antibody blockade. The same receptor-ligand interactions are important for anti-cancer and anti-viral immune responses and the study of viruses has identified new families of immune receptors/ligands. Human cytomegalovirus (HCMV) is the leading infectious cause of congenital birth defects. Indeed, 3 out of 10 babies born each day will have long term problems resulting from HCMV infection. Additionally, HCMV infection causes severe disease in individuals who are immunosuppressed following an organ transplant or living with HIV/AIDS. There is no currently licensed vaccine against HCMV and existing antivirals have problems due to toxicity and development of resistance. HCMV has a large genome encoding approximately 170 canonical genes. HCMV is a paradigm of viral immune evasion. The majority of its genome is dispensable for growth in cell culture systems and is predicted to encode immune evasion genes. Our recent work has identified many novel immune evasion gene functions (1-4) but there are still many genes with no assigned function. HCMV has a number of identified interactions with inhibitory pathways (e.g. 5-6) . This project aims to identify the functions of these 'orphan' genes through screening approaches, involving reporter assays, natural killer cell and T-cell assays and targeted CRISPR/Cas9 technology. 1. Identification of specific inhibitory pathways targeted by HCMV HCMV genes which are present in regions with clear effects on NK and T-cell activation from previous assays and that are expressed on the cell surface from proteomics will be focussed on. The proteins encoded buy these genes will be expressed in different cell systems and as soluble forms for protein purification. CRISPR/Cas9 activation systems will be used to identify interacting receptors using an epithelial cell background.

	Existing in-house and custom reporter constructed for this project will be used to screen existing libraries of HCMV block deletion mutants and adenoviruses expressing individual HCMV genes (4). This aim will identify mechanisms of 'orphan' HCMV gene function. 2. Validation of cellular and HCMV protein-protein interactions Protein-protein using Fc fusion proteins of receptors of interest with wildtype and mutant viruses and tetramer staining with soluble HCMV proteins. Surface plasmon resonance experiments will also be carried out to determine the affinity and kinetics of the interactions. Modelling, mutagenesis and crystal structure determination will follow. This aim will validate the interaction and seek to map functional regions. 3. Functional effects of the interaction NK and T-cell assays with specific mutants including CD107 assays and expansion assays will determine the functional effect of the interaction. Phenotypic characterisation in HCMV seropositive and seronegative individuals will also be carried out. 1. doi: 10.1073/pnas.2309077120 2. doi: 10.1073/pnas.1720950115. 3.DOI: 10.7554/eLife.22206 4.DOI: 10.1371/journal.ppat.1004058 5. DOI: 10.1016/s1074-7613(00)80529-4 6. DOI: 10.3389/fimmu.2017.00298
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