Project Details	
Project Code	MRCIIAR26Ca Fielding
Title	Systematic investigation of the interaction of human cytomegalovirus with the immune system
Research Theme	IIAR
Project Type	Wet lab
Summary	Human cytomegalovirus (HCMV) is a herpesvirus that causes significant disease in individuals with impaired immune control. HCMV causes significant morbidity and mortality in transplant patients and is the major infectious cause of congenital birth defects. It is a paradigm of immune modulation, targeting many immune cells such as natural killer (NK) cells and T-cells. This project investigates how HCMV evades NK cells and unconventional T-cells like gamma delta T-cells. The student will be part of an excellent research team and receive cutting-edge training in virology and immunology.
Description	Immune cells are important for the control of viral infections. In particular, gamma delta T-cells 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 leave alone healthy cells or kill virus-infected cells they encounter. 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 directed against HCMV and existing antivirals have problems due to toxicity and development of resistance [1]. HCMV is a paradigm of viral immune evasion [2]. HCMV has a large double-stranded DNA genome (236 kbp) encoding approximately 170 protein-coding open reading frames (ORFs) [3]. Only 45 of these ORFs are essential for replication of HCMV in vitro, the rest have functions required for clinical infection with many having defined roles in evading the immune response [4]. The ability of HCMV to modulate the immune system allows the virus to persist for life in immunocompetent individuals. Gamma delta T-cells are unconventional T-cells, which express gamma delta T-cell receptor (TCR) chains distinct from conventional alpha beta CD4+ and CD8+ T-cells [5]. They generally respond to host cell proteins upregulated or stabilised during bacterial or virus infections [6-9]. Gamma delta T-cells expressing a delta chain other than V delta 2 (V delta 2 negative) are expanded in HCMV positive individuals and are associated with better prognosis [10-11]. Therefore, gamma delta T-cells are potentially useful in immunotherapy against HCMV. Two fundamental questions in gamma delta T-cell research are (i) what are the ligands recognised by gamma delta TCRs? and (ii) how these ligands are regulated? A greater understanding of both these research questions is needed to understand the 'rules' of gamma delta T-cell recognition

	and to identify their target ligands to enable a rationale for the use of these TCRs in CAR-T immunotherapy for cancer and viral diseases. Aims 1. Characterisation of Annexin-A2 triggered of a gamma delta TCR Annexin-A2 (ANXA2) upregulation during cellular stress was found to trigger the 73R9 gamma delta TCR [8]. In collaboration with the group of Professor Julie Dechanet-Merville (University of Bordeaux), we identified a widespread induction of ANXA2 reactivity by different HCMV mutants using the 73R9 TCR JRT3 Jurkat reporters. This upregulation was previously shown to be dependent of induction of reactive oxygen species (ROS) [8], although the precise mechanism for triggering this pathway during HCMV infection is unclear. We will map the HCMV gene products responsible for triggering this pathway by expressing a library from adenovirus vectors if infection with other viruses (e.g. SARS-CoV-2) suggesting may form a pan-virussensing pathway. 2. Characterisation of a novel gamma delta T-cell immune evasion strategy encoded by HCMV A second HCMV reactive gamma delta TCR, MAU, recognises cell surface expression of the Ephrin A2 receptor (EPHA2) [9]. Our data demonstrates that EPHA2 is downregulated during HCMV infection [12]. However, analysis of HCMV 'block' deletion mutants revealed that deletion of separate region of the genome upregulated activation of the JRT3 MAU reporter. This additional requirement on the cell surface of the infected target cell may contradict the current understanding of recognition by this TCR. Further experiments are needed to determine the mechanism used by HCMV to downregulate ligands and the functional consequences of the effect using activation assays with gamma delta T-cell clones (CD107a mobilisation and IFN and TNF production). 3. Identification and characterisation of novel gamma delta T-cell reactivities Gamma delta T-cells will be expanded using established co-culture assays and our cohort of healthy HCMV seropositive donors. PBMC from healthy individuals will be co-cultured with fibroblasts infected with wildtype HCMV and HCMV 'block' deletion mutants. Expanded gamma delta T-cells will be sorted and analyzed for their phenotype and TCR usage using single cell RNA sequencing. TCR reactivities will be characterisation using cell surface proteomic datasets from the 'block' deletion mutant library and confirmed by CRISPR/Cas9 knockout/siRNA knockdown/blocking antibodies. **References** 1. PMID: 34168328 2. https://doi.org/10.3390/pathogens14070629 3. PMID: 15105547.Lead Supervisor Name Affiliation College/Faculty Department/School Email Address Co-Supervisor 1 Name Affiliation College/Faculty Department/School Co-Supervisor 2 Name Affiliation College/Faculty Department/School Co-Supervisor 3 Name Affiliation College/Faculty Department/School 4. PMID: 14623981. 5. PMID: 39361750

	6. PMID: 24703779 7. PMID: 22885985. 8. PMID: 28270598 9. PMID: 34330813. 10. PMID: 10330426; 11. PMID: 26054538; 12. PMID: 28186488
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