Project Details	
Project Code	MRC21IIRCa Wang
Title	Generating killer cells for immunotherapy against cancer and pathogens
Research Theme	Infection, Immunity & Repair
Summary	Natural killer cells (NK) and CD8+ cytotoxic T lymphocytes (CTL) protect us by killing cells infected with intracellular pathogens and cancers. This project aims to determine pathways that drive the growth of types of NK and CTL that are optimised to provide better protection from these diseases. We will use state-of-the-art technologies, unique libraries of viruses (adenovirus and cytomegalovirus) and clinical samples (leukaemias) as systems of analysis.
Description	This study aims to generate new understanding/reagents that will enable the expansion of highly cytotoxic NK and CD8+ CTL for immunotherapy. CD57 is a carbohydrate antigen found on NK/T-cell subsets and large granular leukaemias (NK-LGL, T-LGL) [1]. Neither the function of CD57, nor the proteins that carry it are known, but it is a signature for highly cytotoxic cells that have been used in immunotherapy. Human cytomegalovirus (HCMV) is a herpesvirus that induces large CD57+ NK and CTL expansions in vivo [2]. Wang/Stanton have worked on HCMV for ~20 years [3-4], generating unique reagents to dissect immune responses to the virus. Stimulating blood-derived cells with customised HCMV vectors enables the in vitro expansion of different immune subsets: deletion of viral genes US2-11 or US18-22 expand CD57- cells, whereas deletion of RL10-UL1 expands CD57+ cells. Thus, manipulation of HCMV genes enables the expansion of cell types capable of enhanced cytotoxic activity. Further, Heurich-Sevcenco has developed biochemical methods for analysing CD57 expressing proteins and Wooldridge grows CD8+ T-LGL clones and has access to NK-LGL. We intend to: 1. Identify individual HCMV genes within US18-22 and RL10-UL1 that expand CD8+CD57- and CD8+CD57+ CTL respectively and define mechanism of action. 2. Perform the same analysis for NK cells targeting expansion of CD57+ NK cells. These are powerful mediators of antibody (Ab)-dependent cellular cytotoxicity direction of which is responsible for many successful cancer treatments. 3. Compare proteins that carry CD57 on CTL, CD57+ NK cells and LGLs and define cell-specific function. In this way, we will define the pathways that drive the growth of different NK and CTL subsets, which can then be exploited to expand optimised effector cells for multiple different therapeutic settings, as well as determine why CD57 is a marker for such effective cells. METHODS Expansion assays will be used to compare HCMV knock-outs (KO) vs wildtype infected cells. Individual HCMV KOs within US18-22 have already been made/analysed by proteomics [5]. Responding cells will be defined by cytometry; CD3, CD8, tetramer, CD45RA, CD45RO, CCR7 (memory); CD57, PD1, Tim3, LAG3 (exhaustion/senescence), CD27, CD95 (stem cell memory), CD56, NKG2C (NK cells). Further expts will use NK and CTL in established proliferation/functional assays. Proteomics (established in Cardiff, collaborators Cambridge [6]) comparing wildtype and HCMV KO infected cells will be used to identify host proteins targeted by particular genes that may orchestrate effector cell expansion. Hits will be validated in the above assays using inhibitory reagents and mechanism defined via

	established techniques. In parallel, proteomics will be used to identify proteins carrying CD57, comparing HCMV-specific T-cells, NK cells, NK-LGL and T-LGL. Hits will be validated biochemically and functionally. REFERENCES [1] Wang E et al. (1995) CD8highCD57+ T lymphocytes in normal, healthy individuals are oligoclonal and respond to human cytomegalovirus. J Immunol 155:5046. [2] Kreutzman A et al. (2011) Expansion of highly differentiated CD8+ T-cells or NK-cells in patients treated with dasatinib is associated with cytomegalovirus reactivation. Leukemia 25:1587. [3] Patel M et al. (2018) HCMV-encoded NK modulators: lessons from in vitro and in vivo genetic variation. Front Immunol 9: e2214. [4] Wang E et al. (2018) Suppression of costimulation by human cytomegalovirus promotes evasion of cellular immune defenses. PNAS 115:4998. [5] Fielding C et al. (2017) Control of immune ligands by members of a cytomegalovirus gene expansion suppresses natural killer cell activation. eLife 6:e22206. [6] Weekes M et al. (2014) Quantitative temporal viromics: a new approach to investigate host-pathogen interaction. Cell,157:1460. [7] Hansen S et al. (2011) Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature 473:523.
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