Project Details	
Project Code	MRC22IIARCa Wang
Title	Generating killer cells for immunotherapy against cancer and pathogen
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Natural killer cells (NK) and CD8+ T cells protect us against intracellular pathogens and cancers. This project aims to determine pathways that can drive the growth of particular types of NK and T cells that are optimised to provide better protection from disease. This knowledge will aid the development of immunotherapies using such effector cell types to target many different diseases. We will use human cytomegalovirus and leukaemic cells as systems of analysis.
Description	This study aims to generate new understanding and reagents that will enable the expansion of highly cytotoxic NK and T-cells for immunotherapy. CD57 is a carbohydrate antigen, that is found on NK and T-cell subsets, and large granular leukaemias (NK-LGL and T-LGL) [1]. Neither the function of CD57, nor the proteins that carry it are known, but it is a signature for highly cytotoxic effector cells that are proposed for use in immunotherapy. One major problem is that such cells do not seem to last in patients after they have been grown outside the body. Human cytomegalovirus (HCMV) is a herpesvirus that induces large NK and CD8+ T-cell expansions expressing CD57 in vivo [2]. Wang/Stanton have worked on HCMV for 20 years [3-4], generating unique reagents and technologies to dissect NK and T-cell responses to the virus. Screening with a new assay shows that stimulating blood-derived cells with customised HCMV vectors drives the in vitro growth in numbers of different T-cell subsets. Deletion of the viral genes US2-11 or US18-22 expand CD57- cells, whereas deletion of RL10-UL1 expands CD57+ cells. Thus, manipulation of HCMV genes in these regions enables the expansion of particular T-cell types. Furthermore, 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+ T cells respectively, and define the underlying molecular mechanism of action. 2. Perform the same analysis for NK cells targeting expansion of CD57+ NK cells (powerful mediators of antibody-dependent cellular cytotoxicity and proposed for use in treating cancers that can be targeted by antibodies). 3. Compare proteins that carry CD57 on NK-LGL, T-LGL, HCMV-specific T-cells, CD57+ NK cells and define function. In this way, we will define the pathways that drive the growth of different NK and T-cell 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 The novel expansion assay will be used to compare HCMV knock-outs (KO) vs wildtype infected cells. Individual HCMV KOs within US18-22 have already been made and 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 CD8+ T-cell lines in established proliferation/functional assays. Proteomics (established in Cardiff, with collaborators in

	Cambridge [6]) comparing wildtype and HCMV KO infected cells will be used to identify host proteins targeted by particular genes that thus 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 (ALL CAPS from applicants) [1] WANG E et al. (1995) J Immunol 155:5046. [2] Kreutzman A et al. (2011) 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) Nature 473:523.
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