Project Details	
Project Code	MRC23IIARCa Wang
Title	Generating killer cells for immunotherapy against cancer and pathogens
Research Theme	Infection, Immunity, Antimicrobial Resistance and 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 work out what drives the growth of types of NK and CTL that are optimised to protect against these diseases, using state-of-the-art technologies (proteomics, advanced flow cytometry), unique libraries of viruses (adenovirus and cytomegalovirus) and clinical samples (leukaemias) 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 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. Indeed, CD57+ NK cells are powerful mediators of antibody-dependent cellular cytotoxicity proposed for use in treating cancers targeted by antibodies, while CD57+ T-cells have been used for immunotherapy against viruses such as human cytomegalovirus (HCMV - see below). One major problem is that such cells do not last in patients after they have been grown outside the body. 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 immune responses to the virus. Screening shows that stimulating blood-derived cells with customised HCMV vectors drives the in vitro growth of different T and NK cell subsets. Block deletion of the viral genes US18-22 expand CD57-, whereas deletion of RL10-UL1 expands CD57+, cells. Thus, manipulation of HCMV genes in these regions enables the expansion of particular T-cell and NK cell types. Furthermore, Heurich-Sevcenco has developed bespoke methods for analysing CD57 expressing proteins and Wooldridge grows CD8+ T-LGL and NK-LGL. We will: 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. 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 An expansion assay will be used to compare HCMV knock-outs (KO) vs wildtype infected cells. Individual HCMV KOs within the US18-22 and RL10-UL11 regions have already been made and some 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 = 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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