Project Details	
Project Code	MRC21IIRCa Stanton
Title	Enhancing immune stimulation of novel viral vaccine vectors
Research Theme	Infection, Immunity & Repair
Summary	HCMV is one of the most promising vaccine vectors for inducing immune responses against pathogens and cancer. However, it's unclear how these responses are induced. We will combine cutting-edge proteomics with functional immunology to determine how HCMV induces such strong responses, enabling us to generate optimised vaccine vectors.
Description	Human cytomegalovirus (HCMV) is an exciting vaccine vector, capable of protecting against both HIV & TB in macaque models. It remains the only vector to ever achieve this impressive feat. Its efficacy arises from its ability to induce unique T-cell responses; it induces the highest number of T-cells of any vector, and these are polyfunctional effector memory T-cells that actively migrate to infected tissues and control infection. Such properties are also highly desirable in cancer vaccines, and we are developing HCMV vectors to induce anti-tumour T-cells. Although the efficacy of HCMV vectors is impressive, the virus is a pathogen, and 'safe' versions have not been generated. Furthermore, how such effective T-cell responses are induced is unclear. If the mechanisms underlying the induction of these T-cells can be determined, the ability could be replicated in other vaccine vectors (e.g. recombinant adenovirus; RAd) that have proven safety records. A potential explanation for the unique T-cell induction by HCMV is that in vivo, HCMV infects the key antigen-presenting cells (APCs) that are responsible for controlling T-cell responses - dendritic cells (DCs), and macrophages. Yet these cells cannot be infected in vitro, preventing characterisation of T-cell induction by HCMV infected APCs. We have made three key breakthroughs that permit us to interrogate this process: •We developed virological systems that enable us to infect DCs and macrophages in vitro, with wildtype virus, and perform functional experiments to characterise virus manipulation of immune-cell function(1). •We developed multiplexed proteomics combined with RNAseq, to determine how the entire proteome of the DC (>8,000 proteins), is affected by infection(2, unpublished). •We have created a bank of HCMV mutants, each lacking a block of ~6 genes. Between these viruses, nearly all non-essential genes are knocked out. Crucially, immune-modulators are generally found in these knocked-out regions. This bank of 'knock-out' viruses is complemented by 180 RAds, expressing each HCMV gene individually. Together these reagents enable rapid screening for loss of function (HCMV mutants) and gain of function (RAds)(3). We have used these techniques to dissect the molecular mechanisms underlying HCMV manipulation of NK and T-cell activation during infection of fibroblasts(4). We will now use them to determine how HCMV manipulates APCs to affect induction of immunity. We will infect DCs and Macrophages with our knock-out viruses, perform functional assessment of the ability of each virus to induce T-cell responses, before using proteomics/RNAseq to determine how each virus manipulates the infected cell. This information will enable us to identify how particular HCMV genes manipulate individual APCs in order to induce T-cell responses that are so much more potent than

	alternative vectors. This understanding can then be used to replicate the potency of HCMV vectors in other widely used vectors. In addition to informing on vaccine development, this will provide information on how HCMV persists lifelong despite the induction of a potent immune response, and the underlying mechanistic basis by which the immune system recognises pathogens in general. In turn, this will identify targets that, if manipulated, can prevent disease caused by multiple different viruses. 1.Murrell et al. The pentameric complex drives immunologically covert cell-cell transmission of wild-type human cytomegalovirus. PNAS. 2017 2.Evans et al. De novo derivation of proteomes from transcriptomes for transcript and protein identification. Nat Methods. 2012 3.Nightingale et al. High-Definition Analysis of Host Protein Stability during Human Cytomegalovirus Infection Reveals Antiviral Factors and Viral Evasion Mechanisms. Cell Host Microbe. 2018 4.Fielding et al. Control of immune ligands by members of a cytomegalovirus gene expansion suppresses natural killer cell activation. Elife. 2017
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