Project Details	
Project Code	MRC22IIARCa Stanton
Title	Investigating the mechanisms, and immunological consequences, of viral cell-cell spread
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Viruses can infect as cell free virus, or transmit directly from cell-to-cell. Cell-cell spread dramatically alters susceptibility to the immune system and therapeutics, yet we have very little understanding of this process. We will use molecular virology, proteomics, and cutting-edge imaging, to work out how cell-cell spread occurs, how it enables viruses to escape immunological and therapeutic control, and how therapeutics might be overcome this problem.
Description	Human cytomegalovirus (HCMV) is a major cause of morbidity and mortality in the immunocompromised, and the leading infectious cause of congenital malformation. It costs healthcare in the USA alone >$4 billion/year. No vaccine is licensed, and antivirals suffer from toxicity and resistance. Inter-host transmission of HCMV is by cell-free virions, and has been widely studied. However intra-host spread occurs by direct cell-to-cell spread. This has not been well studied because laboratory viruses do not undergo this form of transmission. We solved this issue, discovering that cell-cell spread provides protection from humoral immunity, and overcomes innate and intrinsic immunity, potentially explaining the failure of classical vaccine/therapeutic approaches. Furthermore, we identified the viral protein (RL13) that switches HCMV from cell-free to cell-cell mode. This provides a unique opportunity to understand cell-cell spread, and to improve in vivo targeting of HCMV. 1.How can therapeutics be redesigned to target cell-cell spread? Using electron microscopy of cell-cell spreading virus, we observed novel HCMV-driven structures between cells that are reminiscent of virological synapses. These are protected spaces between cells, that allow virus transmission while excluding extracellular molecules (e.g. antibodies). However, the high resolution of EM made finding potential synapses difficult, while the thin sectioning made it impossible to know whether these structures are enclosed spaces. We will therefore use Correlative Light Electron Microscopy (CLEM) to identify synapses by immunofluorescence, before imaging by EM tomography. This will enable high resolution imaging through the complete depth of multiple synapses. We will determine whether they are truly enclosed spaces, or whether molecules with particular properties (e.g. small size) can access them. We will also determine whether virions accumulate within the synapse (and are therefore targetable), or if they simultaneously exit one cell and fuse with the next. This will determine what types of molecules could potentially inhibit cell-cell spread. 2.Which viral/host interactions can be targeted to prevent cell-cell spread? RL13 switches HCMV into cell-cell mode. SILAC-IP of RL13 identified 5 interacting host proteins, which RL13 relocalised to the virus assembly complex (VAC). CRISPR will be used to knock each host protein out, determining which are required for RL13 to drive cell-cell spread, then CLEM will determine effects on virological synapse formation. Given the relocalisation of these proteins to the VAC, we will also use quantitative (SILAC) proteomics of purified virions to determine whether their impact on

	transmission is due to them altering the molecular makeup of virions. This will determine how RL13 causes cell-cell spread, identifying therapeutic targets that could prevent this mode of transmission. 3.Which immunological pathways can be exploited to control cell-cell spreading virus? Restriction factors limit infection, and there is major interest in exploiting them therapeutically. HCMV cell-cell spread increases infection efficiency, particularly in the presence of interferon. I.e. it more readily overcomes innate and intrinsic immunity. However, we do not know whether all factors, or only some, are affected. Antiviral restriction factors will be screened for their ability to inhibit cell-free or cell-cell infection using overexpression, and CRISPR knockout in the presence of interferon. This will define restriction factors that remain effective against cell-cell spread, and can therefore be exploited therapeutically. For those that are ineffective against cell-cell spread, we will investigate why this is. E.g. cell-cell spread delivers high numbers of virions (>500). We will investigate whether this overwhelms restriction factors by varying the ratio/timings of cell:cell interactions during virus spread, to titrate virion delivery.
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