Project Details	
Project Code	MRCIIAR25Br Rivino
Title	Investigating the mechanisms underlying Professional killer cell dysfunction in dengue
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Dengue is a mosquito-borne virus infection now affecting half of the world's population. There is no cure or broadly protective vaccine for dengue. During viral infection, CD8+ T-cells and NK-cells mediate viral clearance by killing virus-infected cells. This project builds on our recent discoveries of a defective killing capacity of T and NK-cells in severe dengue patients. Bridging immunology and virology, the student will identify host and viral factors driving this immune dysfunction in severe dengue. Studies will be performed on dengue patient samples and in-vitro dengue virus-infected cell lines using cutting-edge immunology/virology techniques in a containment level (CL)-3 laboratory.
Description	**Background & preliminary data** Dengue virus (DENV) co-circulates as four serotypes (DENV1-4) and causes symptoms ranging from uncomplicated febrile illness to life-threatening severe dengue (SD) characterized by plasma leakage, haemorrhage and hypovolemic shock. Host immunity plays an important although poorly understood role in dengue pathogenesis. Genetic studies identified single nucleotide polymorphisms in MICB(1) and KLRK1(2) genes encoding for molecules involved in regulation of CD8+ T/NK-cell cytotoxicity, suggesting a potential role for T/NK "professional killer" cells in SD. Accordingly, our recent unpublished data in a Vietnamese dengue cohort shows strong and specific associations of SD with transcriptional/phenotypic T and NK-cell signatures. These signatures of SD which are present early in dengue infection, prior to development of SD, include: (i) T-cell co-expression of multiple co-inhibitory receptors (IRs), (PD-1/PDL-1, Tim-3, TIGIT, LAG-3) and decreased expression of cytotoxic mediators granzyme B and perforin; (ii) poor NK-cell activation and cytotoxicity and NK-cell expression of IRs (LILRB-1, NKG2A, PDL-1, TIGIT, LAG-3). Our preliminary data in dengue patient samples shows that PD-1/PDL-1 blockade may restore the anti-viral function of CD8+ T-cells in dengue. This data suggests that therapies modulating CD8+ T and/or NK-cell responses may be effective for SD. What is causing T and NK-cell expression of IRs and which of these receptors plays a major role in T/NK-cell dysfunction remains unclear. DENV infection was shown to upregulate expression of Galectin-3, the Tim-3 ligand(3); Fielding (Cardiff) showed that SARS-CoV-2 infection downregulates NK-cell activating ligands(4). These data suggest that viruses exploit modulation of T/NK-cell inhibitory/activating ligands for immune evasion. In this project the student will define the IRs causing NK and T-cell dysfunction in dengue and the viral factors that induce their expression in DENV-infected cells. **Key research questions** (i) What is the impact of IR expression on the function of T and NK-cells in dengue? (ii) Does DENV infection induce upregulation of IR ligands in myeloid cells, and by which mechanisms?

	Aims and objectives Aim 1: Impact of IR blockade on CD8+ T-cell function in dengue. Using peripheral blood mononuclear cells (PBMCs) from dengue patients recruited by collaborator Yacoub (OUCRU, Vietnam) the student will perform in-vitro blockade of IR signalling and evaluate whether this restores CD8+ T-cell function. The student will use existing blocking antibodies/drugs (e.g., anti-PD(L)-1, anti-Tim-3) and identify novel strategies to interfere with IR signalling. CD8+ T-cells will be evaluated for their cytotoxic potential (CD107a expression), production of anti-viral cytokines (e.g., IFN-gamma, TNF-alpha and MIP-1beta and direct killing capacity using high-dimensional spectral flow cytometry (Cytek-Aurora) and IncuCyte imaging. Objective 1: Determine CD8+ T-cell function (killing and cytokine production) before and after IR blockade in dengue PBMCs. Aim 2: Impact of NK-cell immunomodulation on NK-cell function in dengue. The student will test whether targeting inhibitory/activating receptor-ligand pairs restores NK-cell function in dengue patient-derived PBMCs/NK-cell lines. As above, existing blocking/activating antibodies and novel strategies will be used to modulate NK-cell activation. The student will optimise novel NK-cell killing assays using target cells relevant for dengue for e.g., K562 cells stably expressing a replication-deficient GFP-DENV replicon generated by Davidson (UoB), or DENV-infected primary myeloid cells. NK-cells will be analysed for expression of novel cytotoxic molecules critical for NK-cell killing that Humphreys' laboratory (Cardiff) has recently identified. Work with dengue patient samples (Aims 1-2) will be performed in the UoB CL-3 laboratory using PBMCs already stored in this site. Objective 2: Determine NK-cell function (killing and cytokine production) before and after immunomodulation in dengue patient PBMCs. Aim 3: Modulation of IR ligand expression by DENV. (a) Expression of IR ligands will be assessed using flow cytometry in GFP-DENV replicon cell lines (K562, Huh7 and HEK 293) available in Davidson's laboratory. Results will be validated in the wild-type cell lines (without DENV-replicon) before and after DENV infection and in DENV-infected monocytes/monocyte-derived dendritic cells which represent the main targets of DENV infection in-vivo (UoB CL-3 lab). (b) Genes encoding the single DENV proteins will be expressed individually in cell lines and tested for their ability to upregulate NK/T-cell IR ligands. Interactome analyses will be performed using co-immunoprecipitation assays and SDS-PAGE/Western blot analyses to identify the underlying mechanisms of IR ligand modulation by DENV proteins. The student will also test whether homologous proteins from other orthoflaviviruses (Zika virus, Yellow fever virus and West Nile virus) have the same effect. Objective 3: Determine expression of IR ligands before and after DENV infection or DENV protein expression. The student will be able to take ownership of the project and propose/test novel strategies to restore the anti-viral function of CD8+ T/NK-cells (e.g. targeting co-stimulatory receptors or other molecules important for cytotoxicity identified by Humphreys). The student will

	have opportunities to train with experts in immunology and virology (Rivino, Davidson, Humphries, Fielding) and interact with clinical scientists at OURCU (Yacoub and team). References (1) http://doi.org/10.1038/ng.960 (2) https://doi.org/10.1093/infdis/jiac093 (3) https://doi.org/10.1002/rmv.1832 (4) https://doi.org/10.7554/eLife.74489
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