Project Details	
Project Code	MRC22IIARBr Rivino
Title	Identifying the impact of genetic variants of MICB associated with severe and symptomatic dengue virus infection on NK and CD8+ T cell function
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	NK and CD8+ T cells are critical components of anti-viral immunity; they express NKG2D which modulates their functions through binding to the inducible MICB ligand. Polymorphisms in MICB associate with increased susceptibility to severe dengue virus infection. You will investigate the mechanistic basis for the association of MICB polymorphisms and severe dengue by using state-of-the-art immunological, genetic and bioinformatic methodologies and work in a Cat-3 lab.
Description	Dengue virus (DENV) is a mosquito-borne flavivirus that circulates as four distinct serotypes (DENV 1-4) and causes the most prevalent mosquito-borne viral disease of humans affecting 390 million people per year. Dengue infection may cause asymptomatic infection, uncomplicated febrile illness or a more severe life-threatening syndrome characterized by plasma leakage and bleeding. There are no therapeutics for dengue and only a partially protective dengue vaccine is available. The host immune system is believed to play a central role in dengue pathology but the mechanisms driving severe disease remain unclear. NK and CD8+ T cells are critical for recognition and killing of virus-infected cells; they express NKG2D, a type II transmembrane glycoprotein that mediates the activation/function of these cells upon binding to ligands such as the major histocompatibility complex class I polypeptide-related sequence B (MICB). Increased susceptibility to severe and symptomatic dengue associates with the MICB intronic single nucleotide polymorphism (SNP) rs3132468 in a genome-wide association study (GWAS) and replication studies. A second intronic MICB SNP rs3134899 also associates with severe dengue, albeit less strongly. The mechanisms underlying genetic susceptibility to severe dengue remains unknown. Our unpublished research shows altered NK and CD8+ T cell responses in severe dengue and a strong, specific association of phenotypic and transcriptional T and NK cell signatures with capillary leak (a measure of dengue severity) and viral load. We therefore hypothesize that the MICB SNPs associated with severe dengue cause altered activation of the MICB-NKG2D axis leading to impaired NK/CD8+ T cell functions and poor viral clearance. This project will test this hypothesis through the development of MICB engineered cell lines, T/NK cell co-culture systems and analysis of clinical samples. Bioinformatic investigations of available datasets will interrogate the broader links of MICB SNPs with susceptibility to viral infection. Knowledge gained in this study could pave the way for the design of novel therapeutics for dengue and/or identify early markers of progression to severe disease, as well as elucidate critical mechanisms underlying anti-viral immunity. RESEARCH PLAN Aim 1: Investigate MICB expression in engineered cell lines and patient samples. Different cell lines (e.g. HepG2, K562, THP-1) will be engineered to express the MICB SNPs using CRISPR-Cas9 and the expression levels of transmembrane and soluble/exosomal MICB before and after DENV 1-4 infection and DENV infectivity will be evaluated respectively by flow cytometry and ImageXpress Pico Cell Imaging

	microscopy. MICB levels and viraemia will also be evaluated in the serum of acute dengue patient samples by ELISA. Aim 2: Investigate the impact of MICB SNPs on T and NK cell phenotype and function. Suitable HLA-I defective cell lines (e.g. GFP-expressing K562 cells, b2mKO human foetal foreskin fibroblasts (Wang)) will be engineered to express the MICB SNPs. Antigen-driven T cell responses will be evaluated after introducing selected HLA-I molecules in GFP-K562 cells and MICB SNPs into a bank of immortalised skin fibroblasts expressing selected HLA-I molecules (HLA-A2, HLA-A11) using a bespoke adenovirus CRISPR delivery system (Stanton). Modulation of NKG2D expression/signalling and a broad range of T/NK-cell functions (e.g. production of cytokines, cytotoxicity) will be tested in primary NK and T cells from HLA-matched dengue patients and in available NK and dengue-specific CD8+ T cell lines/clones by flow cytometry. Aim 3: Investigate using analysis of available genome-wide common variant and sequence data from the Avon Longitudinal Study of Parents and Children (www.bris.ac.uk/alspac) the frequency of these and other SNPs in adjacent positions within this MICB intronic region and evaluate their broader association with susceptibility to common viral infections/disease.
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