ABSTRACT

Dengue virus (DENV) infection is one of the most important arboviral infections in humans. Severe DENV infection is suggested to be an immunopathogenesis where induction of antiviral immune responses may worsen DENV disease. DENV infects around 100 million people annually in tropical and subtropical regions of the world, however DENV treatment is still only supportive without any specific therapies. Despite prolonged efforts to control or reduce DENV transmission, DENV infection continues to increase with a substantial economic burden in DENV endemic countries.

The sphingosine kinase (SK)/sphingosine 1 phosphate (S1P) axis regulates a wide range of cellular signalling processes including survival and proliferation. Further, the SK/S1P pathway is implicated in many diseases such as cancer, inflammatory disorders, and microbial infections. New roles for the function of SKs isoforms, SK1 and SK2 in different viral infections are emerging. The role of SKs enzymes during DENV infection are not fully defined. In this project, we aimed to investigate the effect of SK1 and SK2 on DENV infection and on DENV-induced immune responses in both *in vitro* and *in vivo* infection models. Induction of type I interferon (IFN) and interferon stimulated genes (ISGs) are important first-line host defences against viral infections, and thus we also here have assessed the role of one important ISG, viperin, against DENV infection.

A chemical reduction in SK1 activity using the inhibitors SKi and SK1-I, prior to DENV challenge reduced DENV infection *in vitro*. DENV infection *in vitro* upregulated the mRNA levels of IFN-β, ISGs viperin, IFIT1, IRF7, CXCL10, and tumour necrosis factor-α (TNF-α) and this response was reduced in cells treated with SK inhibitors. Although the reduced induction of these mRNA's may be due to the reduced DENV replication in the presence of SK inhibitors, these data suggest potential roles for SK1 in regulating DENV infection and DENV-induced innate responses *in vitro*.

Since DENV cannot replicate or cause an infection in mice, we employed a reliable model of

DENV-2 infection in the brain of wild type (WT) mice to investigate DENV replication in the context of the complex *in vivo* host cell responses and specifically to assess the effect of the lack of SKs enzymes on DENV infection *in vivo*. Intracranial (ic) DENV infection induced body weight loss and neurovirulence symptoms that reflects DENV replication in the mouse brain. DENV infection in the mouse brain resulted in an induction of IFN-β and viperin, Ifi27l2a, IRF7, and CXCL10 at early stage and later during DENV infection. Further, DENV infection in the mouse brain caused an infiltration of CD8+ but not CD4+ T-cells. DENV ic infection, however does not alter the SK/S1P axis in the mouse brain and the lack of SK1 had no major effect on DENV infection or DENV-induced innate responses and T-cell infiltration. Taken together, these results indicate that SK1 reduced DENV infection in cultured cells but did not influence DENV replication in the mouse brain.

We also investigated the role of SK2 in DENV-infection. Our results showed that while the genetic lack of SK2 in immortalised mouse cells inhibited DENV infection and dysregulated IFN-β and ISGs responses, chemical inhibition of SK2 activity *in vitro* had no impact on DENV infection. Similarly, the genetic lack of SK2 had no effect on DENV infection and DENV-induced immune responses in the mouse brain following ic infection, as above. The lack of SK2, however did alter the SK/S1P axis independently of DENV infection, with a significant reduction in S1P levels in mouse brain, demonstrating a role for SK2 in regulating S1P levels in this tissue. This reduction in S1P in the brain, however still did not affect the infiltrating CD8+ T-cell level following DENV infection suggesting no role for the SK/S1P axis in T-cell infiltration in the DENV-infected mouse brain.

Our results and prior published studies showed an induction of viperin following DENV infection *in vitro* and *in vivo*. The genetic lack of viperin in primary mouse cells increased DENV infection that was associated with enhanced IFN-β expression *in vitro*. Viperin deficiency however had no major effect on DENV replication following ic infection of mouse brain and no effect on the induction of IFN-β and ISGs or TNF-α. The exceptions to this were mRNA levels for Ifi27l2a and

IL-6 that were both upregulated in response to DENV infection and the lack of viperin further increased mRNA levels for both these factors following ic DENV infection. Histological analysis of DENV-infected mouse brains demonstrated that the hippocampus was the region most affected by ic DENV infection. The absence of viperin did not exacerbate these DENV-induced neuropathies and morphological changes in the hippocampus or CD8+ T-cell infiltration in the mouse brain. In this study, immunofluorescence staining of mouse brain section was undertaken to assess the main DENV target cells in the brain and the cell types expressing viperin. Results were not conclusive but were promising and suggestive of positive staining for DENV and viperin in some sections of the DENV-infected WT mouse brain.

Overall, this study has defined potential roles of SK1 *in vitro* in promoting DENV infection and ISG induction but this is not reflected by responses in the brain. Similarly, a lack of SK2 reduces DENV infection and ISG induction *in vitro* but not in the brain. In both cases, DENV-infection of the brain induces CD8+ T-cell infiltration but does not dysregulate the SK/S1P axis. The important ISG, viperin is anti-viral *in vitro* against DENV but lack of viperin does not affect DENV replication or ISG induction in the brain, with the exception of Ifi27l2a and IL-6. This in particular, may warrant further future examination. The DENV-brain mouse model of infection used in this project can be used to further investigate certain aspects of DENV replication *in vivo*, such as studies to investigate the anti-viral potential of chemical SK inhibitors or S1P analogues on DENV replication in the brain. Additionally, our preliminary staining for DENV and viperin expression in mouse brain establishes a foundation for future work to define the principal DENV targeted cells and anti-viral responsive cells to further define the biological responses to DENV-infection in the brain.