SUMMARY

Dengue virus (DENV) is responsible for one of the most important human viral diseases in terms of geographical distribution and morbidity. The pathogenesis of severe DENV results from a combination of multiple factors that act in concert to promote a dysregulated immune response. Hyperactivity of the complement alternative pathway (AP) is associated with severe forms of DENV disease, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). The AP is constitutively active at basal levels and thus is highly regulated by soluble and membrane-associated proteins, keeping the activity of the AP tightly controlled. Factor B (FB) and factor H (FH) are considered two main regulators of the AP. While FB promotes activation of the AP, FH is the major negative regulator responsible for keeping a fine control of this pathway. The overall goal of this thesis is to gain insights into complement dysregulation by investigation of the induction of FH and FB during DENV infection.

Firstly, different methods were established to detect, identify and quantitate FH and FB mRNAs and proteins that were further applied to study changes in *in vitro* and *in vivo* models of DENV infection. *In vitro* DENV infection showed that FH mRNA was significantly increased in DENV-infected endothelial cells (EC) and macrophages but surprisingly, production of extracellular FH was not. This phenomenon was not seen for FB, with DENV induction of both FB mRNA and protein, or with Toll-like receptor 3 or 4 stimulation of EC and macrophages, which induced both FH mRNA and protein. Further, an imbalance in AP components in the local microenvironment of EC and macrophages was detected, with lower FH relative to FB protein along with increased deposition of the complement component C3b on the surface of DENV-infected cells. These changes are predicted to result in higher complement activity locally on the endothelium, with the potential to induce functional changes that may result in increased vascular permeability, a hallmark of DENV disease. Further using MatInspector software, several IFN-responsive elements along with NF$\kappa\beta$ and STAT binding elements were identified within the human FB and FH promoter regions, suggesting that both factors are stimulated by similar transcription factors. Experimentally it was demonstrated that IFN-β mediates induction of FB and FH mRNA and FB protein in EC in a co-ordinated manner consistent with other interferon-stimulated genes. Finally, this study was extended to investigate the roles of FB and FH in DENV-infected AG129 mice, deficient in type I and type II IFN receptors. An early increase in FB

followed by a decrease at moribund stage was detected in mice with severe DENV. In contrast, FH was decreased during the acute phase of infection and increased at end-stage of severe disease. These DENV-induced FB and FH responses in AG129 mice suggest an initial acute-phase response to activate the AP, followed by excessive complement consumption and hyper-activation of the AP. This latter response is consistent with changes in FB and FH described in DHF and DSS patients. Surprisingly, circulating FB and FH protein levels did not correlate with changes in liver mRNA. Similar Matinspector computational analysis of the mouse FB and FH promoters again showed predicted dependence on IFN responsive elements, NFκβ and STAT transcription factors, with clearly the IFN-independent elements such as NFκβ likely to play a major regulatory role in the responses to DENV described in the AG129 IFN-receptor deficient mouse model. DENV-infection in the brain of immunocompetent mice induced FB but not FH mRNA, demonstrating discordant regulation of these genes in this setting that could be mediated via transcription factors such as NFκβ.

Altogether this study suggests potential roles of the AP complement cascade in DENV disease and has provided *in vitro* and *in vivo* evidence of a dysregulation of the AP during DENV infection that can be mediated by changes in FB and FH. This in turn could expand strategies for developing therapeutics to prevent or control the increased vascular permeability and treating the severe forms of DENV disease.