HuVIM Final Report

The project HuVIM aimed to develop and implement a humanized mouse model for the study of meningococcal septicemia. Meningococcal septicemia is a rapidly progressing, potentially fatal disease, which occurs when the bacterial pathogen *Neisseria Meningitidis* gain access to the blood stream. The human specificity of the pathogen *N. Meningitidis* has hampered the use of animal models to study this infection. The aim of this project was to introduce human dermal microvessels into a mouse model though grafting of human skin onto immunocompromised mice.
The project objectives as outlined in the application were as follows:

1. To design and develop a humanized animal model for the study of *Neisseria meningitidis* infection.
2. To use this model to address the following:
   I. Identify mechanisms by which *N. meningitidis* can bind to human endothelium *in vivo*.
   II. Define how infection affects the integrity of human blood vessels *in vivo*.
   III. Investigate the cell signalling pathways initiated following bacterial attachment and the subsequent inflammatory/immunological response.

This project achieved all stated goals and deliverables in a timely and efficient manner.

1. The humanized mouse model we proposed was based on the grafting of human skin onto SCID mice. We sourced human skin from primarily plastic surgery procedures carried out at Hopital Europeen Georges-Pompidou. The grafting procedure proved to be replicable and stable, with a very high (>90%) graft success rate.
2. Implementation of the model to study meningococcal sepsis was also achieved and we were able to address all the outlined objectives. The major results are described below.
   I. *N. meningitidis* adhered rapidly and exclusively to the human vessels in the skin graft when introduced intravenously into the model. The bacteria adhered both as individuals and eventually as microcolonies of various sizes (Figure 1B). By using a library of mutants we were able to show that this binding relied on the Type IV pili of the bacteria.
   II. The pathology of the human skin graft following infection mimicked that seen in human patients. Thrombosis, inflammation, vascular leakage and even the purpuric rash were all identified. This is the first example of purpuric rash development in a model of *N. meningitidis* (Figure 1C).
   III. We were able to identify human cytokine signaling following infection of the model. Using a cytometric bead array (CBA) we could differentiate inflammatory signaling from the human endothelium as opposed to the circulating mouse cells. Human IL-6 and IL-8 were both shown to be significantly upregulated within 24h of infection (Fig 1D).

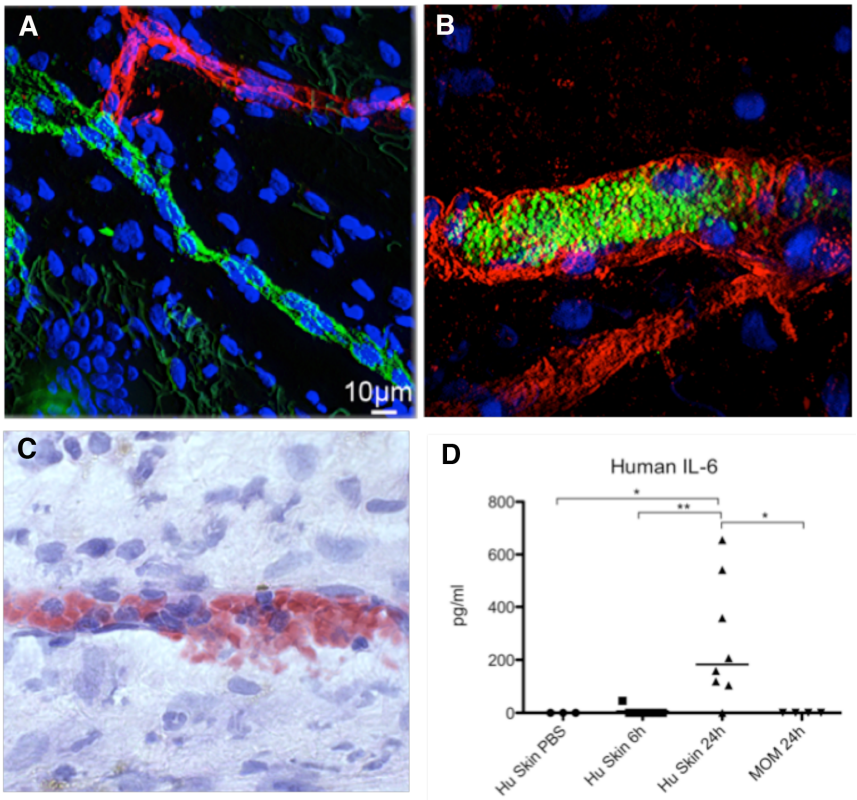

Figure 1 .(A) Mouse vessels (green) joining a human vessel (red) in the edge of the human skin graft. (B) *N. meningitidis* (green) filling a human vessels (red) in the graft. (C) Histology of an infected vessel showing congestion, inflammation and vascular leakage. (D) CBA data showing an upregulation of human IL-6 in the serum on infected grafted mice 24 h post infection.

The project resulted in 4 published papers and an additional paper that is currently 'in press'. The success of the project enabled us to extend the project into other areas of interest such as coagulation and immune cell responses to the infection. Other members of the host laboratory will now continue this preliminary work.

The final results of this project will hopefully give a better understanding of the pathophysiology of early *N. meningitidis* infection.
The results from this project will be of great interest to researchers not only within the *Neisseria* field but also those working with other pathogens that target the blood vessels, and particularly those which display human specificity. It is hoped that this model can be adopted as a standard tool for *Neisseria* infection and can be used to verify and extend the vast amount of research that is currently carried out *in vitro* on cell lines. This model is the first animal model to mimic the development of dermal lesions following infection with *N. meningitidis*. Understanding how the pathogen behaves *in vivo* will be crucial to the development of new drug targets. It is hoped that this work may lead to better treatment strategies and therefore outcomes for patients with meningococcal sepsis.