ImmunoTools *special* Award 2014

Alisha McLoughlin, PhD student

Supervisor: Dr. Phil Cummins

Endothelium Biology Group, School of Biotechnology, Dublin City University, Dublin 9, Ireland

Disruption of Blood-Brain Barrier phenotype by *Staphylococcus aureus* infection: An In-vitro HBMvEC model

Staphylococcus aureus (*S. aureus*) is a gram-positive, cocci-forming bacterium implicated in numerous infections in humans and animals. *S. aureus* is a commensal micro-organism found in the nasal cavity, under-arm, groin and the gastrointestinal tract of the human body. Approximately 20% of the population are carriers of nasal *S. aureus* but they do not experience any problematic symptoms associated with it. Methicillin-resistant *S. aureus* (MRSA) is one of the most prevalent forms of this pathogen commonly occurring in hospitals in immune-compromised patients where it can cause mild to severe infections such as systemic infection post-surgery, skin and soft tissue infections (SSTI), sepsis and meningitis.

In 2010, it was the number one cause of bacterial deaths in the USA. Moreover, *S. aureus* is one of a few bacteria that are able to gain access to the central nervous system via the BBB along with the gram-negative bacteria *Neisseria meningitidis* and gram-positive *Streptococcus pneumonia*. The BBB comprises a monolayer of unique brain microvascular endothelial cells, which act as a seal to separate the central nervous system from the main systemic system. These BMvEC are a key component of the neurovascular unit, which as a whole regulates neurovasculature networks and cerebral homeostasis. These cells are equipped with specialised adherens and tight junction proteins that are found on the transcellular membrane surface as well as intracellularly. These junctional proteins regulate the paracellular entry and exit of molecules that can permeate through to the neural microenvironment. However, when this barrier is compromised by injury and/or infection this can result in BBB dysfunction.

My PhD project sets out to comprehensively model the impact of *Staphylococcus aureus* infection on the barrier phenotype of primary-derived human brain microvascular endothelial cells (HBMvEC) at the molecular and cellular level in vitro.

Cultured HBMvEC are infected with a formaldehyde-fixed or live Newman wild-type *S. aureus* strain with varying bacterial dose (multiplicity of infection/MOI) and infection time, in order to assess tight/adherens junction disruption characteristics. A key component of this project is the bacterial induction of HBMvEC cytokine production. We aim to measure cytokine protein expression by western blotting and ELISA. The use of the IL-6 and TNF-α ELISA set would offer a great insight into determining the cytokines expression profiles. Through flow analysis, we want to assess the viability of the cells post-infection. An apoptosis assay using FITC conjugated anti-human Annexin V would determine this. Positive controls will be required for the aforementioned experiments, therefore with the use of the human recombinant IL-6 and TNF-α, we would have the appropriate controls to standardise the results. Future research includes immune-precipitation assays, NFκB activation as a result of TNF-α and finally examining key virulence factors of *S. aureus.*