

## TITLE

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Identification of novel therapeutic targets for pathogenic inflammation in pneumococcal meningitis.

## SUPERVISORS

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## AIMS

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1. We will use an in vitro transwell model of the human endothelial blood-brain interface to investigate the molecular determinants of innate immune endothelial activation and neutrophil transmigration during *Streptococcus pneumoniae* infection in order to identify potential therapeutic targets in the immunopathogenesis of pneumococcal meningitis.
2. We will use this model to test the potential of anti-inflammatory therapies in current clinical practice to modulate endothelial activation and neutrophil transmigration resulting from innate immune responses to *S. pneumoniae* infection, in order to identify candidates for extended pre-clinical evaluation and subsequent clinical trials.

## BACKGROUND

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*Streptococcus pneumoniae* infection is one of the commonest causes of bacterial meningitis in the UK, associated with a mortality rate of 30% and significant morbidity, with up to 40% of survivors having chronic disability, despite appropriate antibiotic therapy<sup>1,2</sup>- thought to be due to the inflammatory response to infection. The hallmark of *S. pneumoniae* meningitis is an inflammatory exudate with a predominant neutrophil infiltrate<sup>3</sup>. Whilst the physiological function of this host response is to eradicate the microbial pathogen, encapsulated pneumococci can evade neutrophil phagocytosis<sup>4,5</sup> and release of proteolytic enzymes and reactive oxygen or nitrogen intermediates from neutrophils can cause substantial tissue damage. Hence there has been extensive interest in the application of anti-inflammatory therapies for adjunctive treatment of bacterial meningitis. Despite the plethora of new anti-inflammatory drugs targeting specific components of immune responses that have been applied to treatment of inflammatory arthritides, inflammatory bowel disease, multiple sclerosis and periodic fever syndromes<sup>6-9</sup>, only non-specific corticosteroids have been evaluated in clinical trials of bacterial meningitis. There is a need for detailed pre-clinical evaluation of the potential benefits of novel anti-inflammatory agents in a model of *S. pneumoniae* meningitis to identify which will be the most appropriate for use in future clinical trials.

Neutrophil extravasation involves a complex sequence of distinct interactions with endothelia<sup>10</sup>. Proinflammatory cytokines and chemokines augment these interactions and stimulate cell motility. These pro-inflammatory signals predominantly arise from innate immune cellular activation in response to recognition of pathogen associated molecular patterns by germ line encoded host pattern recognition receptors. Endothelial cells do not show significant capacity to generate innate immune inflammatory responses directly. Instead, these responses are best characterised in mononuclear phagocytic cells, particularly macrophages that form resident sentinel cells of the immune system in all tissues<sup>11</sup>.

We propose that the immunopathogenesis of *S. pneumoniae* meningitis is partially due to innate immune host-pathogen interactions between bacteria and mononuclear phagocytic cells of the CNS, which lead to activation of endothelial cells and consequent recruitment of neutrophils. Identification of the molecular mechanisms in this cascade will provide new targets for therapeutic intervention.

## PLAN

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### ***Endothelial activation & neutrophil transmigration in response to macrophage innate immune responses to *S. pneumoniae****

We will first use an *in vitro* transwell model with human cells to test the hypothesis that macrophage innate immune responses to *S. pneumoniae* lead to endothelial activation and neutrophil transmigration across an endothelial barrier. Endothelial activation in our model, will be assessed by whole genome expression profiling, supported by quantitative (q)PCR and protein assays for cell surface markers and soluble mediators of inflammation within the cell culture media on both apical and baso-lateral sides of the endothelium. In the first instance, we will conduct experiments with monocyte derived macrophages, the human brain capillary endothelial cell line hCMEC/D3 and wild type encapsulated *S. pneumoniae*. In order to obtain greater insight into the role of selected microbial components that are either associated with bacterial virulence or represent key components that trigger innate immune responses, we will repeat these experiments with isogenic strains of *S. pneumoniae* that are either deficient for capsule or have exaggerated capsular thickness, and strains that are deficient in cell wall lipoproteins. These experiments will allow us to test the hypothesis that determinants of bacterial virulence, such as capsule, and triggers for macrophage innate immune responses, such as lipoproteins, cause enhanced neutrophil transmigration in our model and are associated with quantitative or qualitative differences in endothelial activation.

### ***Identification of candidate mediators of endothelial activation and neutrophil transmigration.***

Validated responses to pneumococci by macrophages and endothelial cells within the transwell model will be compiled from the experiments described above and subjected to bioinformatic analysis by gene ontology associations for biological processes, molecular function and cellular compartment. Factors that are identified as immune or inflammatory responses, having cytokine or chemokine activity, associated with cell adhesion or the cell-membrane, or being secreted will be selected for further analysis. The effect of specific targeting for selected molecules on neutrophil transmigration in our model will be tested by neutralising antibodies, small molecule antagonists or RNA interference. These experiments will identify specific molecular targets for inhibition of neutrophil recruitment in pneumococcal meningitis that may have therapeutic potential.

### ***Evaluation of selected immunomodulatory agents in clinical use.***

Finally we will evaluate selected immunomodulatory therapies that are currently licensed for human use, for their effect on endothelial activation and neutrophil transmigration in our transwell model. In these experiments we will include corticosteroids, anti-TNF therapies (etanercept and adalimumab), IL-1 receptor antagonist (anakinra), anti-IL6 antibody (tocilizumab), anti- $\alpha$ 4 integrin (natalizumab) and anti-CD11a subunit of LFA-1 (efalizumab). We will therefore be able to identify currently licensed therapies that merit further evaluation in pre-clinical studies and subsequently trials for adjunctive treatment of pyogenic meningitis.

## REFERENCES

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