Establishing the critical role of peripheral blood vessel colonisation by *Neisseria meningitidis* in invasive meningococcal disease

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As a bacterial species, *Neisseria meningitidis* is specialised in the colonisation and infection of humans exclusively. Its narrow host range has long presented challenges to *in vivo* pathogenesis studies due to the dearth of suitable animal models that replicate human meningococcal disease. As a result, there are fundamental gaps in our knowledge with regard to septicaemia and meningitis caused by *N. meningitidis*.

The pathogen is a common inhabitant of the nasopharyngeal tract but elicits disease in only a small percentage of carriers [1, 2]. It is believed that host factors are partly responsible for differences in susceptibility to meningococcal disease across the human population, but there are also strains of the bacterium that exhibit heightened levels of virulence [3, 4].

Once the bacterium enters into the bloodstream, it may multiply leading to an increased risk of sepsis in patients and also penetration of the blood brain barrier and the development of meningitis. Bacterial load per mL of blood has been shown to correlate with meningococcal disease severity, however, the absence of an extended prodromal period means that clinical symptoms can occur abruptly in patients following the initial bacteraemia [5].

Resolving more fully the steps or drivers that lead from meningococcaemia to sepsis and/or meningitis is therefore crucial for improving our understanding of invasive meningococcal disease and for developing timely and more effective medical interventions.

In their paper, “Peripheral blood vessels are a niche for blood-borne meningococci”, published in this issue of *Virulence*, Capel, Barnier, Coureuil and their colleagues from the French National Institute of Health and Medical Research (INSERM), describe an elegant set of experiments in which they applied a model using severe combined immune deficiency (SCID) mice that were grafted with human skin [6]. In this humanised mouse model, the vasculature of the graft is retained enabling the investigators to elucidate specific interactions between meningococci and human endothelial cells during blood-borne infection *in vivo*.

The principal biological questions that the authors explored in this paper included the following:

(i) Is *N. meningitidis* colonisation of human endothelial cells a prerequisite for sepsis and lethality *in vivo*?

(ii) Which *N. meningitidis* genes are required for colonisation of human endothelial cells?

With regard to the first part of the study, the authors inoculated both grafted and non-grafted SCID mice intravenously with $5 \times 10^6$ colony-forming units (CFU) of a virulent, capsulated and pilated serogroup C strain of *N. meningitidis*, 2C4.3. This experiment generated a clear result. At 48 hours post infection, 87.5% of the grafted mice had died compared to 12.5% of the non-grafted mice. Bacteraemia, measured in terms of CFU per mL of blood, was approximately 2 orders of magnitude higher in the grafted mice than in their non-grafted counterparts.
To determine whether adhesion to the endothelium was required for the pathogenesis observed in the grafted mice, the authors ran parallel experiments with a non-adhesive piliated pilC1 mutant derivative of strain 2C4.3. The type IV pilus-associated protein, PilC1, has previously been shown to be required for N. meningitidis adhesion to human endothelial cells [7]. Here, the pilC1 mutant derivative of 2C4.3 was unable to colonise the graft capillaries.

Furthermore, the authors reported that the bacterial load per mL of blood in grafted mice infected with the pilC1 mutant dropped by approximately 3 orders of magnitude at 18 hours post inoculation while all mice infected with this strain survived over the course of the experiment. Hence, the authors conclude that adhesion to human endothelial cells is a key step in the pathogenesis of N. meningitidis that leads to increased levels of bacteremia and also host mortality [6].

Other investigators have previously shown that N. meningitidis grows in human blood. For example, in their work on the transcriptome of N. meningitidis, Echenique-Rivera and colleagues reported a 2-fold increase in N. meningitidis CFU/mL in human whole blood over a 90 minute incubation from a starting bacterial concentration of approximately 10^8 CFU/mL [8]. Similarly, Hedman and co-workers reported growth of N. meningitidis in human whole blood using an inoculum of approximately 10^7 CFU/mL [9].

While the ability of N. meningitidis to grow in human blood may be responsible for a large part of the increase in meningococcal numbers during meningococcaemia, in Figure 1 of their paper in this issue of Virulence, Capel et al. provide evidence that growth of N. meningitidis on human endothelial cells may occur in vivo.

Previous work published by Mairey and co-workers also pointed to this possibility. They found that reduced shear stress associated with very low blood flow, such as that present in capillaries and microvessels, is required for initial attachment of N. meningitidis to endothelial cells in a PilC1-dependent manner [10]. In addition, they reported that the pathogen accumulated on human umbilical vein endothelial cells (HUVECs) in vitro and formed microcolonies that resembled bacterial aggregates observed on blood vessel walls in an infant case of fulminant meningococcal sepsis [10].

Using time lapse video microscopy, Soyer and colleagues in their paper showed N. meningitidis growth as a microcolony on HUVECs in vitro (Movie S1 from [11]). Furthermore, growth of the microcolony to approximately 10 meningococci resulted in accumulation of the linker protein, ezrin, at the plasma membrane followed by actin polymerisation. This triggered the production of multiple 100 nm wide protrusions from the endothelial cell which are believed to help shield N. meningitidis cells from shear stress [11]. Formation of the microcolonies and protrusions was dependent on type IV pilus-associated protein PilV, again emphasising the critical role played by pilus-mediated adhesion in downstream colonisation of blood vessels.

The extent to which growth of N. meningitidis on endothelial cells, with respect to growth in blood, contributes to meningococcal disease progression remains to be determined, but it is worthy of further in-depth investigation and it will be interesting to see future findings in this research space.

In the second part of their study, Capel et al. screened a transposon insertion mutant library of N. meningitidis to identify meningococcal factors that are involved in blood vessel colonisation in vivo [6]. The library was constructed previously by the authors in N. meningitidis serogroup A strain Z5463 using Enterophage KanR-3, a kanamycin-resistance gene containing derivative of bacteriophage Mu [12]. Grafted and non-grafted SCID mice were inoculated with 10^7 CFU of the N. meningitidis transposon mutant library and bacteria were recovered from blood samples at 4 and 18 hours post infection. Transposon insertion sites for input and output libraries were determined using capture by hybridisation combined with next-generation sequencing. Approximately 500 transposon mutants were found to have a greater than log, fold change in fitness in at least one of the conditions tested.

The authors categorised the list of mutants with respect to genes required for early and late colonisation of the human graft, and for growth or survival in the bloodstream. This analysis identified 68 genes that were required for early colonisation (at 4 hours post infection) of the graft blood vessels among which type IV pilus-related genes were a prominent class. 195 genes were identified as being required for late colonisation (at 18 hours post infection) of the graft and included genes encoding classical virulence factors such as the capsular polysaccharide, lipo-oligosaccharide, chorismate biosynthesis, as well as iron and amino acid uptake systems. While most of the latter group of genes were also needed for survival and growth in the bloodstream of non-grafted mice, interestingly, approximately 27% were required for graft colonisation, and not for growth in the blood. This highlights differences in N. meningitidis adaptations to the human endothelial wall and bloodstream habitats.

Conversely, the authors reported that 36% of the genes involved in growth of N. meningitidis in the blood of mice were not required to colonise the human graft. The majority of these genes were involved in metabolic processes including biotin and thiamine synthesis. The authors suggest that the blood vessel niche may be able to provide the pathogen with these nutritional factors in
Further research is warranted to define the role of endothelial cells in supplying essential nutrients to support the growth of *N. meningitidis* during infection.

In conclusion, the authors have developed a unique and powerful experimental platform for the study of meningococcaemia *in vivo*. Based on their results, they have advanced a model for invasive meningococcal disease in which colonisation of human endothelial cells by *N. meningitidis* is a key step in the development of sepsis. They have provided evidence of growth of the pathogen on blood vessel walls which, combined with observations from earlier studies, adds weight to this site being important as a niche for meningococcal multiplication. In addition, they have pinpointed specific *N. meningitidis* genes that enable the pathogen to succeed in this host environment. Hence, it is expected that the authors’ findings will impact the design of follow-up and complementary works, some of which may ultimately support the translation of new candidate biomarkers or tools for the control of invasive meningococcal disease.

**Conflicts of interest**

The author declares no financial or other conflicts of interest.

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