A humanized model of microvascular infection

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“Understanding how *Neisseria meningitidis* causes these vascular injuries during septic shock requires an animal model to mimic this form of the infection.”

Animal models are a cornerstone of infectious disease research, invaluable to investigating how the infectious process progresses and damages the host tissues. Their use has led to great advances in research, treatment and prevention. However, a number of pathogens are human specific, limiting the use of animal models. These pathogens include HIV, HBV, parasites such as the malaria-causing *Plasmodium falciparum* and bacterial pathogens including *Haemophilus influenzae*, *Listeria monocytogenes*, *Salmonella typhi* and *Neisseria meningitidis*. These infections represent a serious socioeconomic problem worldwide and are the focus of much ongoing research.

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The human tropism of these pathogens depends on specific interactions between the host and pathogen and as our knowledge increases, the number of these species-specific steps is growing. A classic example is the interaction between an adhesin and its host receptor, such as in the case of internalin-A and E-cadherin for *L. monocytogenes* [1]. Interactions with the immune system can also generate species specificity. Infection with *S. typhi* provides an interesting illustration. Unlike humans, mice express TLR11, which provides protection against *S. typhi* infection. *TLR11*-knockout mice become as susceptible to the infection as humans [2]. In the case of *N. meningitidis*, our pathogen of interest, several steps of the infection process have been identified as human-specific. First and foremost, type IV pili (Tfp)-dependent adhesion

is species-specific; secondly, iron scavenging by *N. meningitidis* requires human transferrin [3] and thirdly, the complement evasion pathway is human-specific [4].

Research teams over the world have encountered this issue of species specificity and there has been a significant push towards the development of ‘humanized’ models for these infections. Rodent models hold a significant cost and accessibility advantage over models such as primates, which have historically been used in the study of HIV, for example [5].

Humanized rodent models that are currently in use in infectious disease research include the human immune system (HIS) mice, which are created by introducing either human hematopoietic progenitor cells or human peripheral blood cells into immunodeficient mice. HIS mice of various types have been used to study infection with HIV [6] as well as bacterial pathogens such as *S. typhi* [7]. Transgenic mice engineered to express human proteins have proven useful when the factor involved in pathogen interaction is known (e.g., E-cadherin to study invasion by *L. monocytogenes*) [8]. Another approach involves the grafting of human tissue onto immunodeficient mice. Human liver chimeric mice, made by transplanting human hepatocytes, have been used for hepatic pathogens such as the hepatitis viruses and the malaria parasite *P. falciparum* [9,10]. Transplantation of fetal human intestine under the skin in immunodeficient mice has been used to study a number of enteropathic pathogens including *Salmonella* [11], *Shigella* [12] and enterohemorrhagic and enteroaggregative *Escherichia coli* [13,14].

Developing an animal model for *N. meningitidis* infection raises some specific difficulties. For instance, *N. meningitidis* is responsible for two clinically different diseases that need to be addressed separately. *N. meningitidis* causes septic

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shock and meningitis, which can occur either separately or concomitantly. While meningitis is the more well-known of these two diseases, meningococcal sepsis is more deadly, with a fatality rate of up to 52%, and is responsible for 90% of fatal cases [15]. The nonblanching petechial and purpuric rashes that appear on the skin of the patient are hallmarks of meningococcal sepsis and are believed to be the result of extensive damage to the vasculature in the skin. Histological and pathological data collected from the dermal lesions of patients show bacterial aggregates closely associated with capillaries. Infection is linked to activation of the clotting cascade, thrombosis, congestion and stasis within the microvessels [16,17]. Endothelial cell breakdown, vascular leakage and infiltration of immune cells into the area are also observed [15,18,19]. Understanding how *N. meningitidis* causes these vascular injuries during septic shock requires an animal model to mimic this form of the infection.

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Although Tfp-dependent adhesion is generally thought to be an important step in the infection process, the cellular receptor for this bacterial organelle remains to be identified and, at this stage, a knock-in strategy cannot be envisioned. As an alternative, an approach based on tissue grafting was chosen. Human skin, containing an abundance of dermal capillaries, is grafted onto immunodeficient mice [20]. This model was initially developed to study graft rejection [20]. This results in functional human microvessels connected to, and perfused with, the mouse circulation. *N. meningitidis* introduced intravenously into this model becomes rapidly and exclusively associated with the human skin graft. Closer inspection showed that bacteria could be found within the human capillaries [21]. No bacteria were found in similar sized mouse vessels in the local area. This model, with the presence of the human endothelium allowing for extensive infection, highlights how subtle differences between human and mouse cells can make a huge difference in pathogenesis.

Areas that contained bacteria also showed immune cell infiltration and extensive vascular damage, with thrombosis, congestion, endothelial damage and vascular leak. Approximately

30% of infections resulted in visible purpura on the grafted skin. This pathology closely mimicked that seen in clinical patients and represents the first time we have been able to model the development of the purpuric rash. The chimeric nature of the model allowed for the detection of human cytokine upregulation, specifically IL-6 and -8, which was associated with the infected endothelium [21].

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The role of Tfp in the development of the dermal lesions was also investigated. A mutant lacking Tfp showed no accumulation in the human skin graft, no significant inflammatory or vascular pathology and no identifiable human cytokine signaling. These data suggest that the adhesion properties of the Tfp mediate local bacterial adhesion, which is crucial to the development of the dermal lesions. It has previously been thought that circulating bacteria and endotoxin led to the extensive microvasculature damage seen in meningococcal disease. These findings are important to the understanding of the disease mechanisms, especially because they establish a strong link between bacterial adhesion and vascular damage.

This model represents a new tool to study meningococemia in human skin and allows researchers to test a number of *N. meningitidis* infection hypotheses, previously shown *in vitro*, in an *in vivo* setting. This model is malleable from both the host and pathogen aspects, allowing flexibility in terms of both bacterial strains and the environment into which they are introduced. For instance, an extensive library of *N. meningitidis* mutants has been described, some with well-defined *in vitro* phenotypes that would be of great interest to test *in vivo* [22]. On the host side, murine immune cell populations and vascular components can be manipulated with antibodies and pharmacological agents, while the immunodeficient background would allow for the allogenic transfer of human immune cell populations. This concept of combining ‘humanized’ models (i.e., human tissue and HIS) has been previously suggested for the liver chimeras and could also be applied to the skin model [5].

In terms of clinical applications, using this model to test ways to pharmacologically block

bacterial–host interactions could prove to be an exciting new treatment option for meningococemia. Interfering with adhesion and preventing the development of the dermal lesions could lead to far better outcomes in terms of tissue necrosis, debridement and amputations, which are common in meningococemia survivors. Another exciting prospect is to use these new humanized models to test the efficiency of vaccine targets. Currently the lack of *in vivo* models for meningococcus means there is no preclinical animal model for new vaccine candidates. Although the mouse model used in the skin graft model is immunodeficient, it would be possible to use antibodies generated in immunocompetent mice to test their bactericidal activities, especially their activity on adherent microcolonies in the vessels.

Furthermore, it would now be of interest to extend these findings beyond *N. meningitidis*

and investigate the importance of local endothelial adhesion in other bloodborne infections. The role bacterial adhesion plays in vascular damage and purpuric rash development in other sepsis-causing pathogens can now be addressed. Therefore, this skin graft approach adds to the toolbox for researchers studying deadly infections targeting the human vasculature.

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