Managing meningitis

By Tracey Baas, Senior Editor

SHX Vaccines is taking a new approach to vaccinating against meningitis—preventing meningococci from binding to host cells in the first place. The company's university collaborators have shown proof of concept for the approach in human tissue explants, including efficacy against serotype B—the bacteria's most common but most intractable subgroup.¹

The next step is finding the *Neisseria meningitidis* epitopes that interact with their newly identified human receptor—CD147 (basigin Ok blood group; BSG; EMMPRIN).

N. meningitidis is a Gram-negative bacterium that exists exclusively in humans. The microorganism typically resides in the nasopharyngeal mucosa, where it has no effects. Problems arise when meningococci enter the bloodstream, at which point the bacteria can disseminate to various tissues and result in potentially lethal meningitis.

N. meningitidis serogroup B is a common cause of bacterial meningitis and sepsis in infants in Europe and in the U.S.

Licensed meningococcal vaccines use a polysaccharide fragment found in the bacterial outer coating of serogroups A, C, W and Y to induce a protective immune response. However, serogroup B's outer coating resembles human neural cell tissue and therefore does not induce an immune response, thus precluding a polysaccharide vaccine strategy.

There are two vaccines for serogroup B in registration, and both have breakthrough therapy designation from the FDA. The vaccines—LP2086 from **Pfizer Inc.** and Bexsero from **Novartis AG**—use isolated protein fragments found on the outer surface of serogroup B bacteria that can induce an immune response. Bexsero is approved in Europe, Canada and Australia.

In April, **GlaxoSmithKline plc** announced its purchase of Novartis' vaccine business, with Bexsero being part of the deal.

Stéphane Hugué, CEO of SHX Vaccines, said that it is unlikely that Bexsero or LP2086 will be sufficient to provide protection from all B strains. In addition, patients still would require a different vaccine against the other serotypes.

Now, a French team has developed a different strategy—inhibit any serotype of *N. meningitidis* from adhering to human endothelial cells in the first place.

The first step was finding the precise cellular adhesion receptor that interacts with meningococcal pili. A series of cell line studies pinpointed

CD147 as the receptor. CD147 has broad tissue distribution and surface exposure, and its expression is enhanced at sites of meningococcal adhesion.

In human endothelial cells, CD147-specific siRNA or an anti-CD147 mAb decreased meningococcal adhesion compared with control siRNA or a control protein. Results were consistent when using meningococcal strains belonging to serogroups A, B, C and W.

The next order of business was finding out which bacterial pilus components interacted with CD147. A cell-based competition assay showed that *N. meningitidis* major pilin PilE (pilE) and *N. meningitidis* type IV pilus assembly protein (pilV) were the culprits.

Indeed, mutant meningococci that lacked pilE or pilV failed to adhere to human endothelial cells.

To investigate the role of pilE and pilV in colonization *in vivo*, the team used humanized SCID mice engrafted with human skin containing functional human blood vessels. The model allows the study of meningococci infection because the bacteria only colonize human cells and not mouse cells.

In the mouse model, wild-type meningococci massively colonized the human dermal vasculature, whereas mutant bacteria that lacked pilE or pilV failed to colonize.

Similar results were seen using *in situ* meningococcal infection models of fresh human frontal brain tissues obtained from deceased normal individuals. Wild-type meningococci developed microcolonies adjacent to CD147⁺ cells, whereas meningococci lacking pilE or pilV colonized poorly. Pretreatment with the antibody significantly decreased adhesion of bacteria to human brain vessels compared with

pretreatment using control antibodies.

Results were published in *Nature Medicine*.

The sticky details

It remains unclear how pilE and pilV act in concert to initiate bacterial adhesion to CD147.

Ongoing work by the team includes identifying the precise pilE and pilV epitopes required for interaction with CD147. The research could allow the development of pilin-targeted antibodies and lead to new vaccines for meningococcal infection.

Guillaume Duménil, group leader at the **Paris Cardiovascular Research Center**, wanted to see additional experiments in the mouse model. Duménil's laboratory developed the humanized SCID mice engrafted with human skin to study *N. meningitidis* infection.

"The team shows that bacteria lacking pilV or pilE do not colonize the human vasculature, but I would like to see in future experiments that blocking of CD147 also impedes colonization," Duménil noted. "This would have more fully substantiated CD147 as the endothelial receptor for *N. meningitidis*."

"The team's *in vitro* experiments also hinted that CD147 interacts with multimeric forms of the pilins rather than monomeric forms, so

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—Guillaume Duménil,
Paris Cardiovascular Research Center

having a crystal structure of the pilin-CD147 interaction could also be key,” added Duménil. “With a crystal structure in hand, the precise pilE and pilV epitopes required for interaction with CD147 could be determined. Knowing the epitopes would allow sequence homology studies among different serotypes and strains and might give some indication of what type of broad coverage could be achieved using pili-specific antibodies or vaccine strategies.”

David Stephens said that exploring the direct blocking of bacterial pilV or pilE could be attractive as an adjuvant therapy to complement antibiotics.

Standard of care for people who are suspected to have *N. meningitidis* disease is the immediate delivery of intramuscular or i.v. antibiotics and hospital admission. “There have been a lot of failed attempts at adjuvant therapies to block host inflammatory events or host targets in meningococcal sepsis,” said Stephens. “A lot of the failures have to do with the rapid onset of meningococcal sepsis and the inflammatory cascade that has already been launched before reaching medical care.” Stephens is VP for research at **Emory University’s** Woodruff Health Sciences Center, chair of the university’s Department of Medicine and chief of medicine at **Emory Healthcare**.

A prophylactic vaccine that targets a wide range of invasive *N. meningitidis* would alleviate these problems.

“Antibodies that prevent pilus-CD147 interactions could provide a potential novel path to a broad-based meningococcal vaccine that prevents systemic disease,” said Stephens.

“We are sponsoring ongoing work by the Paris team that will determine the specific pili epitopes that interact with CD147 in order to develop

bacteria-specific antibodies for meningococcal serogroups A, C, W and Y, as well as multiple strains of serogroup B,” said Huguet. “Our ultimate goal is to use epitope-antibody structural information to develop vaccines.”

SHX Vaccines was founded in March 2012 to develop antibodies and vaccines for *N. meningitidis* infection.

Findings in the new study are patented by the **Institut National de la Santé et de la Recherche Médicale** (INSERM) and **University Paris Descartes**. The IP is licensed to SHX Vaccines.

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