Positioning properdin

By Kai-Jye Lou, Senior Writer

Researchers at the **University of Leicester** have shown that a recombinant properdin produced in collaboration with **The Medicines Co.** has markedly higher antibacterial activity than the native protein.[1] The researchers are now working to improve the activity and homogeneity of recombinant properdin to treat a broad range of infections.

Properdin is a glycoprotein composed entirely of thrombospondin-like repeat motifs. It is typically found in circulation as a dimer, trimer or tetramer. The protein stabilizes a key enzyme called alternative pathway C3 convertase that amplifies complement system activity.

The complement system is part of the innate immune system and helps promote the clearance of pathogens as well as potentially harmful substances such as cellular debris.

About 3.5 years ago, Wilhelm Schwaeble and his team at the university started a collaboration with The Medicines Co. to develop methods for producing recombinant human thrombospondin proteins in eukaryotic cells. The goal was to produce thrombospondin-1 (TSP-1; THBS1) for studies to evaluate the glycoprotein's effects on platelet activation and strategies to modulate thrombospondin-mediated thrombus formation. Schwaeble is a professor of immunology at the university and a Royal Society Wolfson Research Merit Award holder.

The Medicines Co.'s lead infectious disease program is oritavancin, a semisynthetic, broad-spectrum lipoglycopeptide antibiotic. In February, the company submitted an NDA to the FDA for oritavancin to treat acute bacterial skin and skin structure infections (ABSSSIs) caused by Gram-positive bacteria and an MAA to the EMA for oritavancin to treat complicated skin and skin tissue infections (cSSTIs) caused by Gram-positive bacteria. The company could not be reached for comment and does not have a disclosed therapeutic program involving properdin.

An unexpected result from the collaboration has been the generation of recombinant properdin oligomers that are much larger than those normally found in serum. According to Schwaeble, these oligomers come in a range of sizes and are comprised of more than 12 properdin monomers linked in a head-to-tail fashion.

Previously, larger properdin oligomers had been shown to stabilize the alternative pathway C3 convertase better than smaller oligomers.[2]

In the current study, the researchers sought to assess the activity of the large recombinant properdin oligomers against bacterial pathogens.

In vitro, recombinant properdin was about 100-fold more effective at supporting complement activation than native properdin. The large oligomers also increased complement-mediated lysis of *Neisseria meningitidis* compared with the native protein in serum.

In mice, i.p. injection of the recombinant properdin prior to a lethal challenge with *N. meningitidis* resulted in 90% survival, whereas injection of saline led to 0% survival. Mice treated with recombinant properdin three hours after a lethal *N. meningitidis* challenge showed significantly longer survival than saline-treated controls (p=0.0007).

In mice infected with *Streptococcus pneumoniae*, injection of the recombinant properdin at the time of infection resulted in minimal or no disease, whereas injection of saline resulted in 70% of mice being euthanized because of development of severe disease.

Results were published in the *Proceedings of the National Academy of Sciences*. The Medicines Co. provided support for the study.

"Our recombinant properdin was found to form higher-order polymers with much higher functionality than those typically obtained from properdin purified from mouse and human serum," said corresponding author Schwaeble.

He said that the speed, strength and brevity of the properdin-induced innate immune response are the key benefits of the approach. "This is what we want in an effective immune response—we want it to come on quick and strong to clear the infection and then go away after it has fulfilled its task in killing and/or clearing the invading pathogens and before damaging healthy tissues," Schwaeble told *SciBX*.

"Aside from the positive effects observed in the tested model systems, the lack of an endotoxic shock response is particularly promising," added Dennis Hourcade, a research professor of medicine at the **Washington University in St. Louis School of Medicine**.

> **"If the approach does prove useful in *Pseudomonas* or in other bacteria known to have become highly resistant to antibiotics, then something like this could be promising."**
> ***—Sanjay Ram, University of Massachusetts Medical School***

Endotoxic shock, or septic shock, can be triggered by endotoxins released into the bloodstream.

Indeed, Schwaeble had expected to see a septic shock response in mice because the complement-mediated bacteriolytic activity induced by recombinant properdin would release large quantities of bacterial debris into the bloodstream.

However, he said that the lack of a shock response could be because recombinant properdin potently ramps up complement-mediated clearance of cellular debris.

Active immunotherapy for infections

Schwaeble thinks that the recombinant properdin has potential as an active immunotherapy to treat infections.

He added that although antibiotics act quickly, they do nothing to clear the cellular debris generated from killing the bacteria. Such debris could induce septic shock.

Schwaeble thus said that recombinant properdin has the potential to

be used in combination with antibiotics to help promote the clearance of bacterial debris and mitigate the risks of septic shock.

Sanjay Ram, an associate professor of medicine in the division of infectious diseases and immunology at the **University of Massachusetts Medical School**, said that it is desirable to have active immunotherapies against drug-resistant bacteria.

Although he noted that the emergence of antibiotic resistance in *N. meningitidis* and *S. pneumoniae* in the U.S. has been limited thus far, Ram added, "if the approach does prove useful in *Pseudomonas* or in other bacteria known to have become highly resistant to antibiotics, then something like this could be promising."

Both Ram and Hourcade wanted additional studies to characterize the safety of recombinant properdin given that the complement activation induced by the multimeric form of the protein is nonspecific and has the potential to damage healthy tissues.

Ram said that it will be important to assess the degree of complement activation, cytokine levels and kidney function following multiple doses with recombinant properdin.

Hourcade wanted to see studies to determine whether recombinant properdin overwhelms the effects of human complement factor H (CFH), which protects healthy tissues from complement-mediated attack. He added that it also will be important to evaluate recombinant properdin in settings in which there is a pre-existing autoimmune or inflammatory condition such as arthritis.

Finally, Hourcade wanted to see biodistribution studies of recombinant properdin, including how long the protein remains in circulation, how it is metabolized and how it is cleared from the body.

Schwaeble said that his group is trying to further increase the functional activity of recombinant properdin and produce it under better-controlled conditions and according to pharmacological standards.

"Our goal is to manufacture a more homogeneous and stable product in a large-scale production process," he said.

The group also is evaluating recombinant properdin in additional models of bacterial infection, including *Pseudomonas*, as well as in models of parasitic infection.

The University of Leicester and The Medicines Co. have cofiled a patent covering the use of the recombinant properdin for treating or preventing properdin-related diseases and disorders, such as *N. meningitidis* infection. The work is available for licensing.

Lou, K.-J. *SciBX* 7(14); doi:10.1038/scibx.2014.390
Published online April 10, 2014

REFERENCES

1. Ali, Y.M. *et al. Proc. Natl. Acad. Sci. USA*; published online March 24, 2014; doi:10.1073/pnas.1401011111
   Contact: Wilhelm J. Schwaeble, University of Leicester, Leicester, U.K.
   e-mail: ws5@le.ac.uk
2. Higgins, J.M. *et al. J. Immunol.* **155**, 5777–5785 (1995)

COMPANIES AND INSTITUTIONS MENTIONED

The Medicines Co. (NASDAQ:MDCO), Parsippany, N.J.
University of Leicester, Leicester, U.K.
University of Massachusetts Medical School, Worcester, Mass.
Washington University in St. Louis School of Medicine, St. Louis, Mo.