

MAY 18, 2008

Researchers Find that Mysterious Protein Protects Against Sepsis

A team led by Howard Hughes Medical Institute investigator Jamey D. Marth has shown that a prominent protein on the surface of liver cells protects organisms during sepsis, one of the most common and deadly side effects of bacterial infection. Their finding could help pave the way for new therapies to treat the condition, which causes widespread inflammation and blood clotting, and affects 18 million people worldwide each year.

Marth, who is at the University of California, San Diego (UCSD), and his colleagues published their findings in an advance online publication of the journal *Nature Medicine* on May 18, 2008. Their work reveals the role of a molecule that has mystified scientists for decades, demonstrating that it helps the body fight off the excessive blood clotting associating with severe sepsis, which leads to death in a significant percentage of cases.

More than 35 years ago, researchers discovered a protein that is present in vast numbers on the surface of liver cells, or hepatocytes. These Ashwell receptors -- named for their principal discoverer Gilbert Ashwell, a biochemist who works at the National Institutes of Health -- are common in all vertebrates. In the vertebrates that have been studied, hepatocytes express half a million or more Ashwell receptors shortly after birth.

"These findings contradict the prevailing notion that the low platelet count of sepsis is due to the consumption of coagulation factors caused by the pathogen and is therefore harmful."

- Jamey D. Marth

The receptors enable liver cells to quickly bind and remove specific types of glycoproteins from the bloodstream. Glycoproteins are proteins that have sugar linkages (glycans) attached to them. Ashwell showed that the receptors bind to a specific type of glycoprotein - those that lack a sugar molecule called sialic acid on their glycan chains.

However, scientists had only observed the receptors function in this way when glycoproteins were injected directly into the bloodstream during laboratory experiments. They had never identified a role for Ashwell receptors under normal conditions. In fact, laboratory mice that lack the receptor appear to be perfectly healthy.

Those who study glycoproteins have long been looking for answers to fundamental questions about the Ashwell receptor. “We found that the Ashwell receptor of the liver is poised to rapidly modulate blood coagulation, and then of course we were drawn to understand why should this be,” says Marth. “Why does the liver express five hundred thousand Ashwell receptors after birth? Why are they conserved throughout vertebrate evolution? What critical challenge is the Ashwell receptor awaiting?”

Marth wanted to find out whether there were any glycoproteins that naturally circulate in the blood that might be taken up by the Ashwell receptors. He suspected that the reason none had been found might be that these glycoproteins might be typically “masked” by a particular post-translational modification. So, eight years ago, his laboratory began studying mice that had been genetically modified to that lack the enzymes that made that modification.

They found that eliminating one of these enzymes - a sialyltransferase, which adds sialic acid to glycoproteins -- reduces factors in the blood that are vital for normal clotting. His team has now shown that these factors, a protein called von Willebrand Factor (vWF) and platelets, are targets for the Ashwell receptor.

Marth next considered why Ashwell receptors appear shortly after birth, and recalled that many pathogens, including *Streptococcus pneumoniae* and the influenza virus, remove sialic acids from host glycoproteins to allow them to infect cells. Marth thought that the Ashwell receptor might operate in response to exposure to these pathogens. To test the role of Ashwell receptors during exposure to pathogens, his laboratory infected mice with *S. pneumoniae*. Following exposure, the mice had reduced platelet counts and vWF levels in their blood. Mice without Ashwell receptors, on the other hand, maintained normal platelet counts and vWF levels and had severe clotting that led to tissue damage and organ failure. They also died sooner and more frequently with markedly increased coagulopathy than did mice with normal Ashwell receptors. This shows, Marth says, that the Ashwell receptor mitigates the lethal coagulopathy of sepsis and improves an animal's chance of surviving infection.

Marth says the findings alter previous views about the function of hepatocytes, the coagulopathy by sepsis, and the rationale for treatment during infection. Until now, scientists have thought that the reduction in platelets associated with sepsis was a result of the abnormal blood clotting. “These findings contradict the prevailing notion that the low platelet count of sepsis is due to the consumption of coagulation factors caused by the

pathogen and is therefore harmful,” said Marth. “Rather, this low platelet count is due to the Ashwell receptor and is beneficial by reducing the potential for tissue damage and organ failure, thereby improving the chance of survival.”

Victor Nizet, a UCSD professor of pediatrics and pharmacy and an authority on childhood sepsis who collaborated with Marth on the study, says, “This research provides a whole new way of thinking about coagulation problems in sepsis due to pneumococcus and related pathogens.” He and Marth are now working on developing agents that can engage and enhance the activity of the Ashwell receptor during sepsis and coagulopathy caused by various pathogens in order to increase the chances for patient survival.