Researchers block immune cell rush behind deadly sepsis

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Researchers have found a way to block the ability of white blood cells to sprint toward the sites of infection when such speed worsens the damage done by sepsis, the often fatal, whole-body bacterial infection, according to a study published today in the journal *Blood*. The results recommend existing drugs as potential new treatments against sepsis, and suggest improvements in the current treatment that would increase its effect while eliminating a treatment-related risk for internal bleeding.

A simple bacterial infection becomes sepsis, or "blood poisoning," when it gets bad enough to set off system-wide responses from the body's immune defenses and blood-clotting system. It becomes septic shock when bacteria, the toxins they produce and the body's overwhelming immune response cause multiple organ failure. More than 30 percent of patients with severe sepsis die despite advances in critical care, about 250,000 people per year. Physicians currently rely on antibiotics and surgical drainage, but new options are needed.

White blood cells called neutrophils fight infection by swarming toward bacteria to engulf and destroy them with toxic molecules. Because these same molecules also damage human cells, this phase of the immune response is carefully contained and quickly shut down. The massive rush of neutrophils seen in sepsis, however, can overcome these restraints. In between infections, dormant neutrophils drift with the bloodstream until they "realize" they are passing by the part of a blood vessel wall closest to an infection. Proteins on the neutraphil's surface called integrins then unfold and "grab" the surface of the blood vessel wall, resisting the flow.

The same proteins then help the neutraphil crawl along the tissue scaffold toward the infection site.

In the current study, a team of researchers at the University of Rochester Medical Center demonstrated for the first time that the only approved sepsis drug treatment, recombinant human activated protein C (rhAPC), has its effect by interfering with specific integrins on neutrophil surfaces, which keeps the cells from moving. Importantly, they also learned that a small protein piece of rhAPC, the "RGD" peptide, is responsible for the treatment's effectiveness against sepsis.

"Our results create the distinct possibility that several drugs already approved as safe in humans may have a second use in sepsis," said Minsoo Kim, Ph.D., assistant professor of Microbiology and Immunology within the David H. Smith Center for Vaccine Biology and Immunology at the Medical Center, and lead author of the article. "That is exciting because it could dramatically increase the pace at which new treatments for sepsis arrive in the clinic."

While the standard approach for decades has been to try to kill bacteria with antibiotics, some newer medications are designed to lessen the body's inflammatory reaction to sepsis. Most of these attempts have failed, but Drotrecogin alpha (brand name Xigris® from Eli Lilly), a genetically engineered (recombinant) form human activated protein C (rhAPC), was shown in a recent study to decrease mortality by about six percent, from 31 percent to 25 percent, in severe sepsis patients. Is the only FDA-approved drug for treating severe sepsis and the drug used in the current study.

Before its approval for use in sepsis, rhAPC was known for its ability to prevent blood clots, and researchers thought initially that this ability explained its efficacy against sepsis. When other anti-clotting agents failed to work the same way, however, researchers began looking

elsewhere. Research published by Jerry Nick, M.D., and colleagues at the National Jewish Medical and Research Center (*Blood*. 2004;104:3878-3885) was the first to suggest that the benefit of rhAPC in sepsis might be explained by its effect on white blood cell migration, not blood coagulation, and several papers followed to confirm the idea. Until the current study, however, no one had been able to show how.

Furthermore, the U.S. Food and Drug Administration earlier this month announced that it was analyzing a report just published in the journal Critical Care Medicine that found Xigris, because of its effect on clotting, may increase the risk of dangerous bleeding in patients with a recent history of hemorrhages. The company argues that the study was flawed, and the drug's label is very clear about bleeding risk. Whatever the case, Jiang and colleagues are excited because their results argue that the part of Xigris that contributes to bleeding has nothing to do with its effect on sepsis, and can be removed.

Halting the Great Migration

In a neutrophil at rest, integrins are kept in a "non-stick" state. When the cell gets ready to move, however, integrins are quickly activated on the cell's "foot," the area where the cell touches the surface it wants to move across. Integrins bind to their partner proteins on the surface, and the neutraphil's cell skeleton contracts to pull itself over the leading-edge integrins. Previous studies in Kim's lab suggest that, without precise, integrin-mediated changes that enable the front end to gain traction, and the tail end to let go, immune cells could not migrate. He studied T cells in his earlier experiment, but the current results suggest the same processes are in play in neutrophils. In the current study, the research team showed for the first time that rhAPC has an effect on sepsis because it directly binds to β1 and β3 integrins on the surface of neutrophils and prevents those integrins from grabbing the surface.

Just as importantly, the team proved that human rhAPC contains the "RGD" three-amino acid chain. This peptide is a key component of several human proteins (e.g. fibronectin) over which neutrophils crawl because it has the right shape to be grabbed by integrin. In the case of rhAPC, its RGD chains grab neutraphil integrins first, taking away their ability to gain traction on surfaces. When the current research team changed the shape of the RGD sequence in rhAPC, the medication could neither bind to integrin nor interfere with the migration of neutrophils toward infection sites. In addition, treatment of septic mice with a single dose of the RGD peptide delivered the same improvement in survival as a dose of whole rhAPC, about 30 percent.

Kim's team tracked the ability of neutrophils to migrate across a glass plate coated with fibronectin. The team placed the neutrophils on the surface and then hit them with a type of molecule produced by bacteria, and toward which neutrophils swarm. The results show that, although neutrophils could sense the bacterial product and had a "desire" in chemical terms to move toward it, they could not in the presence of rhAPC.

Along with Kim, the work was led by Pranita Sarangi, Young-min Hyun, Joseph Hollenbaugh and David Topham within the Department of Microbiology and Immunology at the Medical Center, and by Hung-Li Chung and James McGrath in the Department of Biomechanical Engineering.

Gwendolyn Elphick, Alfred Ayala and Jonathan Reichner led the research at the Department of Surgery at Rhode Island Hospital, as did Walter Biffl in the Department of Surgery at Denver Health Medical Center and Alireza Rezaie in the Department of Biochemistry and Molecular Biology at the Saint Louis University School of Medicine. The work was supported by the National Institutes of Health.

"If, as suggested by our results and the literature, the effects of rhAPC on sepsis are attributable to reduced neutrophil migration, then anti-integrin agents represent a new class of drug candidates for sepsis," Kim said. "An RGD peptide already in clinical trials for cancer has potential against sepsis. We are also using molecular biology techniques to look for protein fragments similar to RGD, but with even greater ability to attach to and shut down activated integrins, and having shed the rhAPC anti-clotting functions that create bleeding risk."

Source: University of Rochester Medical Center