(Anti-) Septic Strategies

New drugs hold promise for treating life-threatening bacterial infections

"When in continued fever, the external surface of the body is cold and internally great heat is felt; with thirst, the affection is mortal."
— Hippocrates, 400 B.C.

By CAROL EZZELL

The patient and her doctor thought she had weathered her ordeal.

Even though her appendix had ruptured as she sat at her desk the previous week, forcing herself to work through what she thought was "just a touch of stomach flu," she had reached the hospital in time to have emergency surgery. This morning, five days later, she awoke in her hospital bed feeling a little better, with only some pain around her stitches. Encouraged, she planned to ask her doctor when she might go home.

But soon after lunch, she began to feel cold and clammy. When the nurse took her temperature, he found that it had already climbed to 102°F. Worse, she couldn't remember when she had last urinated, even though she had been through two bags of intravenous fluid since the evening before.

Her head swam as the nurse wrapped a cuff around her arm to check her blood pressure. Through her fuzziness, she saw the nurse write "dropping, 90/60" on her chart and then leave to summon her doctor.

She was going into septic shock.

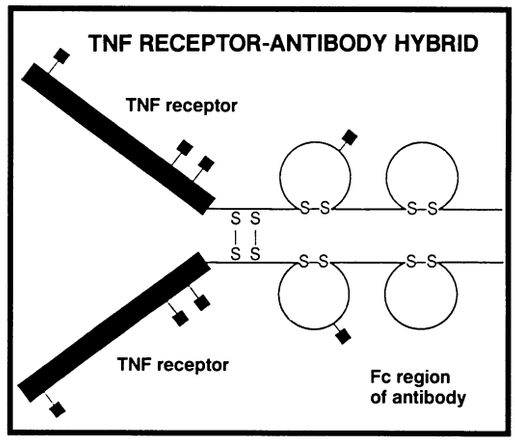
The life-threatening bacterial infections that physicians call sepsis strike approximately two patients every *hour* in U.S. hospitals. The Centers for Disease Control estimates that 100,000 individuals in the United States die of septic shock each year, making it the nation's 13th leading cause of death.

Sepsis takes a high death toll because physicians have no specific, effective treatment for it, says Gordon R. Bernard of the Center for Lung Research at Vanderbilt University in Nashville. At an International Business Communications conference on sepsis held in Philadelphia in June, Bernard noted that physicians currently rely on antibiotics and "supportive therapy" such as intravenous fluids to help patients fight off sepsis.

Roughly half of all sepsis patients die — even if an antibiotic manages to clear the bacteria from the bloodstream. In these people, the body's natural antibacterial response runs amok, leading to the dangerous symptoms of sepsis syndrome. This disorder, described centuries ago by Hippocrates, can culminate in fatal septic shock.

"The originator is gone, but the inflammatory response continues," explains Fletcher B. Taylor Jr. of the Oklahoma Medical Research Foundation in Oklahoma City. "It ends in a real biologic meltdown."

To thwart this death spiral, infectious disease specialists and biotechnology and pharmaceutical companies are working to develop compounds to sop up or inactivate bits of partially destroyed bacteria that can keep the body battling an already eradicated infection. These researchers are also devising new strategies to rein in the body's sometimes fatal over-response to bacteria.



Genentech's sepsis drug, an immunoadhesin, consists of two of the body's own receptors for tumor necrosis factor (TNF) linked to the back end, or Fc region, of an antibody. (S denotes disulfide bonds holding the molecule together.) Once each receptor sops up a TNF molecule, the Fc region targets them for destruction.

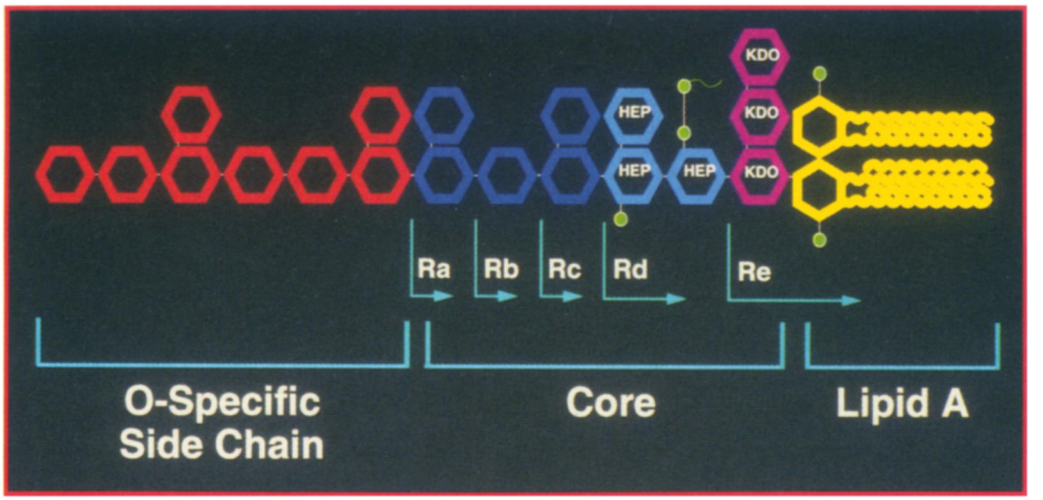
More than two-thirds of septic shock cases result from gram-negative bacteria, named for their inability to retain a particular dark purple stain after treatment with an alcohol or acetone solvent. The solvent bleaches gram-negative bacteria, which microbiologists can then detect using a pink counterstain. Gram-positive bacteria remain purple.

The staining difference highlights a fundamental variation between these two major types of bacteria. The thick outer cell walls of gram-positive bacteria consist of cross-linked sugar molecules, which prevent a solvent from washing away the first stain. But the thinner cell walls of gram-negative bacteria are made of a compound called lipopolysaccharide, which allows the stain to leach out.

It's this lipopolysaccharide, also known as endotoxin, that brings about the rapid deterioration characteristic of sepsis syndrome. When still embedded in the cell wall of an intact bacterium, endotoxin presents little health hazard. But when the immune system or antibiotics attack gram-negative bacteria, they expose a toxic region at the root of the endotoxin molecule. This region, called lipid A, is normally obscured by the molecule's natural twists and turns.

Because lipid A is essentially the same among most species of gram-negative bacteria, drug companies initially sought ways to inactivate it as a possible means of treating sepsis syndrome. The first two drugs — developed by Centocor, Inc., of Malvern, Pa., and Xoma Corp. of Berkeley, Calif. — consist of specific, or monoclonal, antibodies that attack only lipid A.

In recent months, however, both companies have suffered setbacks in their attempts to obtain Food and Drug Administration approval for these drugs. In April, the FDA announced it would not allow Centocor's drug, called HA-1A, on



Xoma Corp.

Schematic drawing of lipopolysaccharide, the endotoxin found in the cell walls of gram-negative bacteria. The deadly lipid A region (yellow) lies at the base of the molecule, where the side chain (red) and core (blue) usually shield it from the body. Once exposed by antibiotics or the immune system, however, lipid A sets off a chain of events that can culminate in septic shock. Ra, Rb, etc., denote steps in the molecule's synthesis.

the market, even though an expert panel convened last September to advise the agency recommended approving the drug.

The FDA rejected the results of two clinical trials of HA-1A sponsored by Centocor on grounds that the company took an early look at the preliminary results and changed the ground rules of the trials in midstream in a way that could have made the drug appear more effective. In June, Centocor announced it would conduct another large clinical trial to prove HA-1A's efficacy.

Last fall, the same FDA advisory committee declined to consider Xoma's drug, known as E5, because the agency hadn't yet reviewed the results of a second large clinical trial of the drug. And in June, the FDA asked Xoma to perform a third trial of E5 because the therapy appeared to benefit only sepsis patients with endotoxin in the bloodstream — a subgroup that the two previous studies were not large enough to assess statistically.

Patrick J. Scannon, Xoma's vice chairman for scientific and medical affairs, says the company is currently attempting to convince the FDA to allow it to pool the data from the two completed trials into a so-called meta-analysis. Combining the data from both trials into this single study, Scannon says, should prove that E5 improves the survival of patients with detectable levels of endotoxin in their blood.

"Sepsis patients have many underlying diseases," Scannon asserts. "Meta-analysis allows you to look and see if there are any meaningful trends" indicating a drug's benefit among subsets of patients with different symptoms, he says.

But while Centocor and Xoma attempt to convince the FDA of the merits of HA-1A and E5, they and several other companies are also developing newer, "second-generation" drugs against sepsis. Many of these target the proteins responsible for the body's runaway response that leads to septic shock.

When white blood cells encounter lipid A, they make a cascade of proteins — such as tumor necrosis factor (TNF), interleukin-1, interleukin-6, and interleukin-8 — that stimulate the immune system. In 1985, researchers found that TNF, which was known to fight tumors in laboratory animals, is identical to a previously identified protein known to cause dramatic weight loss in cancer patients (SN: 8/31/85, p.132). In subsequent experiments, other scientists discovered that simple injections of either TNF or interleukin-1 could elicit the sepsis-like symptoms of fever and chills in healthy volunteers, suggesting that these proteins play a role in the disorder.

That finding prompted several companies to test monoclonal antibodies against TNF as potential treatments for sepsis. At the June sepsis meeting, Mark W. Bodmer of Celltech, Ltd., in Slough, England, reported that treatment with Celltech's anti-TNF antibody completely protected six baboons against an otherwise lethal injection of 40 billion gut bacteria.

The antibody, named CDP571, remained in the bloodstreams of a second group of healthy monkeys for roughly one week without causing an immune reaction, Bodmer and his colleagues observed. They estimate that a single dose would persist in humans for between five and 10 days — long enough, the researchers hope, to stymie the emergence or recurrence of sepsis syndrome. Bodmer says Celltech plans to begin human clinical trials of the antibody in Europe "starting at any time."

Centocor has already demonstrated the safety of its anti-TNF antibody, named cA2, among a small group of healthy volunteers given injections of endotoxin, says Craig R. Smith, Centocor's vice president for clinical affairs. He told the sepsis meeting that Centocor is now launching a large clinical trial in Europe to test the antibody's safety and efficacy among actual sepsis patients. Roughly half of those patients will also receive treatment with Centocor's HA-1A anti-endotoxin antibody, he says.

For their parts, Genentech, Inc., of South San Francisco and Immunex Corp. of Seattle have devised genetically engineered compounds for combating TNF. Both companies are testing hybrid molecules composed of the natural cell-surface receptor for TNF coupled to the back end of an antibody. This pairing creates a larger molecule that takes longer than the receptor alone to break down, allowing the receptor hybrid to persist in the bloodstream for days instead of hours.

Genentech scientist Avi Ashkenazi told the sepsis meeting that in cell culture tests, his company's so-called immunoadhesin sopped up five times more TNF than did an anti-TNF antibody alone. Moreover, a single dose of the immunoadhesin protected 60 percent of a group of mice from lethal injections of endotoxin — even when given three hours after the endotoxin shot.

Immunologist Kendall M. Mohler presented similar results from animal tests of Immunex's TNF receptor-antibody hybrid. Like Genentech's immunoadhesin, the Immunex molecule allowed a majority of mice to survive a lethal dose of endotoxin given three hours previously. However, Mohler reported, nearly all mice died if the treatment was delayed four or more hours after the endotoxin injection.

The similarity between the Immunex and Genentech results "speaks to the general efficacy of TNF receptor-based therapies," Mohler says. Immunex began a clinical trial of its hybrid molecule in July; Genentech plans clinical tests at an undisclosed time.

While Celltech, Centocor, Genentech, and Immunex tackle TNF, researchers at Synergen, Inc., of Boulder, Colo., have set their sights on another body protein responsible for the deadly symptoms of sepsis syndrome: in-

terleukin-1. They have isolated from white blood cells called monocytes a molecule that blocks and inactivates the interleukin-1 receptors on other white blood cells, effectively shutting down interleukin-1's ability to stimulate—and sometimes *over*-stimulate—the immune system.

John P. Pribble, Synergen's assistant director of clinical research, told the sepsis conference that this so-called interleukin-1 receptor antagonist, or IL-1ra, reduced the death rate among a group of 99 patients with sepsis. Only 16 percent of the patients who received the highest dose of IL-1ra died, Pribble reported, compared with 44 percent of those who received a placebo. He said the drug also reduced the average hospital stay of surviving patients by six days.

Xoma and Incyte Pharmaceuticals, Inc., of Palo Alto, Calif., are also developing a naturally occurring protein as a potential sepsis treatment, but theirs—called bactericidal/permeability increasing protein (BPI)—targets endotoxin directly. Both companies assert that BPI constitutes the body's natural defense against endotoxin: Once secreted by a particular type of white blood cell called a neutrophil, it literally pokes holes in the cell walls of gram-negative bacteria, binding to and neutralizing endotoxin.

Paul J. Conlon, director of Xoma's preclinical immunology department, reported at the sepsis conference that a streamlined version of BPI produced by genetic engineering decreased the ability of a mixture of lab-cultured white blood cells to make TNF and interleukin-1, -6, and -8 after exposure to endotoxin. Moreover, pretreatment with BPI saved 90 percent of a group of rats from potentially fatal respiratory infections caused by gut bacteria. Normally, only 10 percent of such animals would survive such an infection, Conlon said.

"We're very excited about these results," says Xoma's Scannon. "This animal model is very similar to gram-negative pneumonia in humans."

Incyte has had similarly positive results with its BPI drug. Charles J. Fisher Jr., director of the critical-care research unit at the Cleveland (Ohio) Clinic Foundation, reported that Incyte's BPI protected 60 percent of a group of rats from otherwise lethal injections of endotoxin. In contrast, he said, none of a separate group of rats treated with Centocor's anti-endotoxin antibody, HA-1A, survived the endotoxin injections.

With the host of new anti-sepsis drugs entering clinical trials—and Centocor's and Xoma's monoclonal antibodies edging toward FDA approval—many physicians are optimistic that sepsis will soon claim far fewer victims. Says Vanderbilt's Bernard, "I'm convinced that within the next few years there'll be a viable treatment for sepsis." □