and rifampicin. In Europe, however, just as in the United States, drug resistant TB is more of a problem in AIDS patients than in the general population.

Still, Jenkins says, the better performance on this side of the Atlantic so far "doesn't mean we can afford to be complacent. [Multi-drug resistant TB] will need constant vigilance because it can creep up on you very quickly." With this in mind, the Communicable Disease Surveillance Centre in north London is tightening up its surveillance methods by introducing a computerized notification system for all cases of drug-resistant tuberculosis in each of the six regional centers for tuberculosis bacteriology.

But even though some drug-resistance patterns vary, one serious worry can be shared equally by health officials on both sides of the Atlantic. That's the emergence of vancomycin-resistant strains of *Enterococci*, which cause urinary tract and wound infections and, occasionally, meningitis, which is rapidly fatal unless checked by antibiotic treatment. "Vancomycin resistance in these bacteria was negligible up to the mid-1980s, but it's taken off in a big way since then," Johnson says. He cites the increase in the number of hospitals in England and Wales that have sent vancomycin-resistant samples to the Antibiotic Reference Unit as an indication of the scale of the problem.

In 1988, only one hospital made such a report, but by 1993 the number had risen to 18. And that's probably just the tip of the iceberg, Johnson notes: "Other laboratories are undoubtedly picking up these strains and not reporting them to us, so the incidence of this type of resistance is probably considerably greater than these figures suggest."

The emergence of the vancomycin-resistant *Enterococci* is worrisome because these bacteria are themselves a significant cause of hospital infections. But even more alarming is the possibility that *Enterococci* will spread vancomycin resistance to other genera of bacteria. Researchers think this will eventually happen because bacteria are very adept at exchanging their antibiotic resistance genes. A particular nightmare is that methicillin-resistant *Staphylococcus aureus* (MRSA), a common cause of hospital infections, will acquire resistance to vancomycin—currently one of the few antibiotics to which MRSA infections will reliably respond. "If the vancomycin resistance gene got into MRSA, that could be potentially disastrous," Johnson says. "You could end up with infections that may be virtually untreatable, and that is a real cause for concern for the future."

–Sharon Kingman

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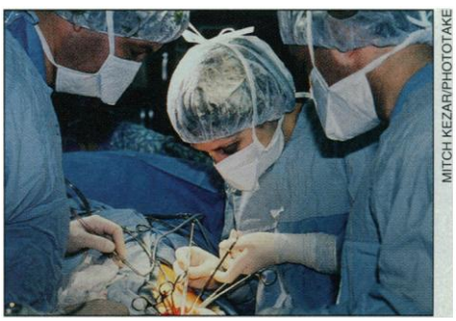
DRUG DEVELOPMENT

Search for Sepsis Drugs Goes On Despite Past Failures

Row after row of stainless-steel vats fill the cavernous halls of Synergen Inc.'s protein manufacturing plant in Boulder, Colorado. The colossal, 82,000-square-foot plant was built to churn out a protein called Antril, which Synergen hoped would be the first successful drug for treating sepsis syndrome, a serious medical condition in which the body's immune system overreacts to an infection and goes haywire (see box, p. 366).

But for now Synergen's gleaming fermenters lie fallow much of the time, because the company encountered a major roadblock in its efforts to bring a potential blockbuster drug to market. To get approval for Antril from the U.S. Food and Drug Administration, Synergen needed good results from a large-scale clinical trial. However, when the trial was completed in February 1993, the news was bad: Antril worked less effectively than smaller, preliminary trials had led company executives to expect. "The failure of the trial was a shock," says Larry Soll, Synergen's chief executive officer. And company officials presumably found it small comfort that Synergen was not alone in its misery. In the previous year, two other firms, Centocor, Inc. of Malvern, Pennsylvania, and Xoma Corp. of Berkeley, California, had seen promising sepsis drugs falter in clinical trials.



MITCH KEZAR/PHOTOTAKE

A risk. Surgery patients and others with wounds are among those likely to get sepsis.

But these failures don't mean the search for an antisepsis drug is over. Far from it. Today, many biotech companies are in hot pursuit of just such drugs—with good reason. "It's not just about money—a lot of people die," says molecular biologist Larry Gold, a Synergen founder who has formed a new company, Boulder-based Nexagen, that's also looking for new sepsis drugs. Gold speaks the truth. Every year in the United States, some 500,000 people come down with sepsis and 175,000 of them die. And, of course, numbers like those translate into other numbers that make the biotechs salivate: The market for a successful antisepsis drug could be $500 million a year, says Jeffrey Casdin, a biotech analyst at Oppenheimer & Co. Inc., of New York City.

What's more, that market may be growing. According to the Centers for Disease Control and Prevention, the number of sepsis cases linked to microbial infections in hospital patients tripled from 1979 to 1992—partly because of the increased vulnerability of the patient population, which includes more older patients and more AIDS patients. Both groups have weakened immune systems that predispose them to sepsis.

This burgeoning market could be a pot of gold for the 20 or so companies now developing possible antisepsis drugs. But, as the experiences of Synergen, Centocor, and Xoma show, the route to this prize is neither straight nor easy. "Even though sepsis is a relatively attractive target for a biotech company, it's also one that everyone has tripped over," says Richard Rose, vice president of drug development for Cytel Pharmaceuticals of San Diego, which currently doesn't have sepsis in its sights. Yet the prize is attractive enough that, no matter how many runners trip, others are there to vie for the inside track.

Preying on the weak. Like a wolf stalking the sickly members of a herd of elk, sepsis finds its victims among the weak, particularly among patients in hospital intensive-care units who come down with infections. All told, about 95% of sepsis cases are caused by infections of some kind; most of the remaining 5% occur, for reasons that are poorly understood, in people with severe injuries but no signs of infection.

Even among the cases caused by infection, there's plenty of variety. While most used to be caused by *Escherichia coli* and other gram-negative bacteria (so-called because they fail to absorb a particular stain), an increasing proportion—now 55% of the total—are triggered by gram-positive bacteria and fungi. "There's been a dramatic shift recently in the pathogens that cause sepsis," says sepsis researcher Roger Bone of the Medical College of Ohio in Toledo.

Despite the dizzying array of microbes that trigger sepsis, researchers in the past decade have learned enough about the molecular biology of the initiating pathogens as well as the immune cascade that causes sepsis to begin identifying targets for antisepsis drugs. "The clinical research done so far has put us

in a position to rationally design new therapies," says Joseph De Angelo, vice president of research for Apex Bioscience in Research Triangle Park, North Carolina.

One of the first targets was endotoxin, a lipopolysaccharide that forms part of the outer cell membrane of gram-negative bacteria. Starting in the mid-1980s, three companies—Centocor, Xoma, and Chiron of Emeryville, California—developed monoclonal antibodies to lipid A, a component of endotoxin that plays a key role in generating the immune attack after it's released from a bacterium. By binding to receptors on immune cells called monocytes and macrophages, lipid A triggers the release of powerful chemical signals known as cytokines. While needed for mounting normal immune responses, some cytokines can contribute to sepsis if produced in excess.

The idea of using antibodies to mop up excess lipid A before it causes the immune response to spiral out of control seemed promising, but it has so far failed to pay off. Xoma and Centocor have seen their products stumble in clinical trials. (Chiron's clinical trial results aren't expected until later this spring.)

One problem that makes it difficult to show that the drugs have a clinical benefit is that the antibodies can help only those patients whose sepsis is triggered by gram-negative bacteria—about 40% of the total. Yet doctors have to give the drug to all patients, because it takes so long to identify the causative pathogen that if they wait for the results before beginning therapy, the patients may die. However, the problems of some of the drugs tried so far may run much deeper than clinical-trial design: Centocor had to halt a human clinical trial of its drug, Centoxin, in January 1993 after a preliminary analysis showed that sepsis patients on Centoxin were dying at a higher rate than those on placebo.

Persevering. But other firms are undaunted by these unsettling results. For example, EntreMed Inc., in Rockville, Maryland, is taking another tack, one predicated on treating the condition before "the cat's let out of the bag," according to Carol Nacy, senior vice president for research at EntreMed. Rather than giving patients antibodies to lipid A, EntreMed is trying to use an "antisepsis vaccine" to get patients to mount their own immune response. The vaccine, discovered by Colonel Carl Alving while working on a lipid A-containing malaria vaccine for the army, consists of lipid A sheathed in vesicles made of lipids and cholesterol. Because lipid A is enclosed in the vesicles, it doesn't trigger cytokine production by macrophages and monocytes, but it still elicits production of antibodies to the lipid in animals, says Nacy, and protects mice against infection by *Escherichia coli*.

Realizing that there could be more than one way to block endotoxin, scientists at Ribi Immunochem in Hamilton, Montana, are trying to develop a benign form of lipid A that can be given to patients to protect them from the effects of endotoxin. They altered lipid A in a way that greatly reduces its toxic effects, although the researchers don't fully understand how. In small-scale human trials, says Ribi spokesman Jeffrey McDowell, the altered lipid A "seems to tolerize the host to the presence of endotoxin" so that the immune system no longer becomes overstimulated by it. As a bonus, McDowell says, the compound triggers a generalized immune response that appears to protect mice against gram-positive bacteria, which don't exude endotoxin, as well as against gram-negative bacteria.

A third anti-endotoxin approach is to try mimic one of the body's own weapons against endotoxin, a substance called bactericidal/permeability increasing protein (BPI), present in certain white blood cells called neutrophils, which binds to endotoxin and takes it out of commission. Two companies—Incyte Pharmaceuticals of Palo Alto, California, and Xoma—have developed recombinant fragments of BPI that bind to lipid A. Both potential drugs have shown

Sepsis: An Immune System Gone Haywire

Like Yellowstone's Old Faithful, the immune system ordinarily works reliably to fight off invading pathogens. But sometimes, like a geyser that keeps spewing hot water long after it should have subsided, the immune system goes haywire as it defends against infectious disease. The result is sepsis syndrome, a condition that kills 175,000 people every year in the United States.

Unlike what happens in sepsis, a normal immune response knows when to rein itself in. It begins when white blood cells called neutrophils squeeze through the blood-vessel walls on a search-and-destroy mission for a bacterial pathogen in the surrounding tissue. The neutrophils kill the bacteria directly by releasing toxic chemicals, such as damaging forms of oxygen and the protein-splitting enzymes elastase and collagenase.

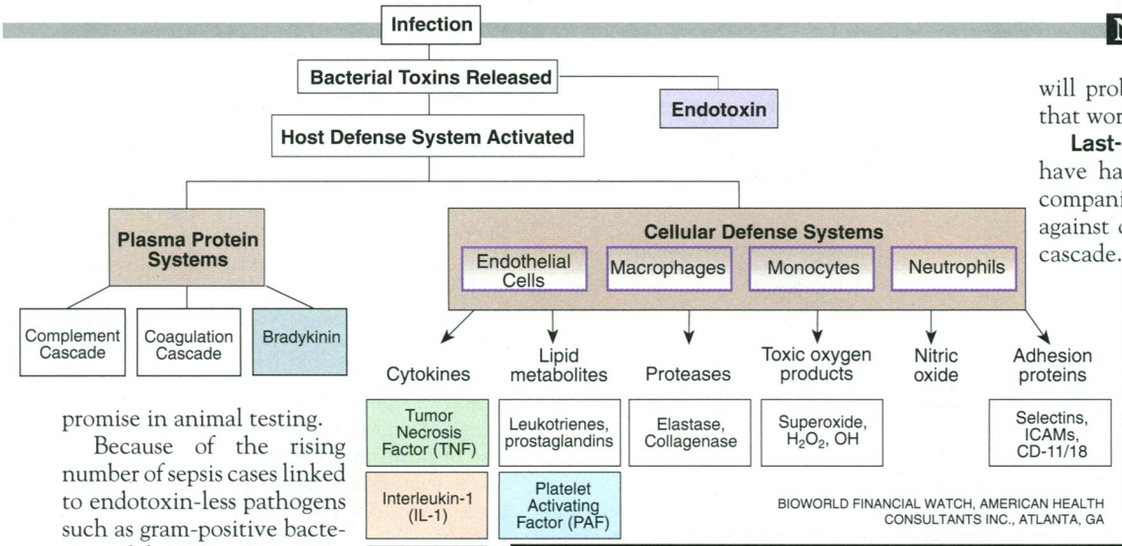
They also draw other immune players to the war zone, among them T cells and antibody-making B cells, as well as macrophages and monocytes, which release powerful immune-response modulators called cytokines. The cytokines add further steam, directly by stimulating immune cell activities, and indirectly by making blood vessels more permeable to the cells flooding into the infection site. Then, as the immune system gets the upper hand over the invading bacteria, other cytokines signal that the battle has been won, bringing the defensive action to an end.

But when that cutoff mechanism fails, which is most likely to happen in AIDS patients and others with defective immune systems, sepsis may begin. Excess neutrophils and macrophages stream to the site of an injury or infection. Immune-stimulating cytokines, whose action would normally be leashed in by other, balancing cytokines, go unchecked, triggering the release of substances that damage the blood-vessel walls to the point where they spring leaks, causing the blood pressure to fall.

Perceiving a persistent threat, even more monocytes and macrophages show up at the damage site and release cytokines. The blood vessels continue to deteriorate to the point where they can't provide a steady supply of nutrients to the body's tissues. The net effect is that whole organs begin to shut down. More than half the patients die if they lose the function of two or more organs. "We can usually control the infection, but we're left with the damage caused by mediator compounds," says Frank Cerra, director of critical care at the University of Minnesota Medical Center.

But by unraveling the pathways that lead to sepsis, researchers have identified a wealth of targets for drugs that might halt the abnormal immune response before it causes permanent damage (see main story). Unfortunately, however, even the most promising efforts haven't produced much improvement in mortality. Researchers find, frustratingly, that capping the geyser at one blowhole seems to cause it to gush out-of-control elsewhere. "When you see a patient, you don't know which cytokines are up and which are down during an often rapidly changing process," says Jack Levin, a hematologist at the University of California, San Francisco. A successful treatment, he says, "strikes me as extremely difficult to achieve." Other scientists, however, see hope on the horizon. "Even though we don't have a slam dunk, it's been a fruitful scientific endeavor," says Roger Bone, president of the Medical College of Ohio in Toledo. "I think we're destined to find a valuable drug."

–R.S.



promise in animal testing.

Because of the rising number of sepsis cases linked to endotoxin-less pathogens such as gram-positive bacteria and fungi, many companies are focusing instead on the immune players that contribute to the development of sepsis of all origins. Two particularly tempting targets are the cytokines interleukin 1 (IL-1) and tumor necrosis factor (TNF), which together play an important role in sepsis; they both raise the body temperature, increase the permeability of blood vessels, and trigger the release of other cytokines, all processes that can push the body toward the organ failure seen in severe sepsis cases. Synergen's Antril, for example, is a protein produced by monocytes that helps counteract IL-1's effects by binding to its receptors on white blood cells. In preliminary trials, Antril sharply cut sepsis mortality, but in the larger trial completed last year Antril seemed to work well only in patients who were already very ill.

That was a disappointing result, but Synergen hasn't given up on Antril. Instead, the company launched a new trial that will focus on the most severely ill patients. "We learned that you must be able to identify in advance segments of the patient population that will benefit from a sepsis drug," says Synergen's Soll. Results of the new Antril trial are expected next year.

Perhaps the largest biotech posse is out to get what appears to be IL-1's main partner in immune devastation, TNF. Several companies, including Chiron, Celltech of Slough, England, Centocor, and F. Hoffman-La Roche Ltd. in Basel, Switzerland, have had encouraging results with TNF-binding antibodies in animal trials and are now testing them in humans.

However, the TNF approach has encountered its own obstacles on the path to a sepsis drug. One tack—to sop up the cytokine with a recombinant form of its own receptor—suffered a setback last September when Immunex Corp. in Seattle, Washington abandoned its recombinant TNF receptor as a potential antisepsis drug after clinical trials showed no benefit. And the news continues to worsen for this strategy: Last month, *BioWorld Today* reported that the Immunex product appeared to increase mortality in sepsis patients.

But the anti-cytokine approach still has plenty of defenders, and some of them argue that the trial results so far have been disappointing because they targeted only one of the chemicals at a time. Take Kent Erickson, a cell biologist at the University of California, Davis, who with his colleagues is attempting to develop an anti-cytokine—in this case a ribozyme (or RNA enzyme) that inhibits TNF production by cleaving the messenger RNA that directs its synthesis.

While the ribozyme reduces TNF levels by up to 90% in mice, Erickson worries that that won't be sufficient to help sepsis patients, since other cytokines, like IL-1, have similar effects. "One of the concerns we have is that if you block TNF, there's enough redundancy in the immune system that IL-1 levels probably go up," he says. Erickson argues that a successful therapy against sepsis will probably require a cocktail of agents that work against both TNF and IL-1.

Last-ditch efforts. Because anti-cytokines have had their share of failures, biotech companies are hoping to make inroads against other components of the immune cascade. Among these are bradykinin, a peptide hormone that triggers pain, swelling, and other signs of inflammation as well as altering blood vessel permeability, and nitric oxide (NO). While bradykinin acts early in the sepsis pathway, NO acts late, coming in after the cytokines which stimulate its production by the blood vessels. NO causes problems because it can react with an unstable form of oxygen to make a damaging compound called peroxinitrite that, like other agents in the sepsis cascade, perforates blood-vessel walls.

Apex Bioscience, for one, is aiming to block NO. Targeting a chemical that acts after the cytokines may not get at the root of the problem, Apex's De Angelo says, but he argues that it "should keep a person alive long enough for the condition to resolve itself through standard treatment."

Sepsis has only become more complex the more researchers study it, which means it's likely that other targets for sepsis drugs will manifest themselves in the coming months. At the moment, says Bone, "we're in our embryonic stage of understanding the pathogenesis of sepsis." In spite of these hopes, the complexity of the disease process, coupled with recent product setbacks, make many biotech firms leery of touting their work on sepsis drugs. Companies are so sensitive that some testing such compounds asked to be excluded from this article; others have opted to stay out of the race altogether.

But for those who are still in, the past failures provide food for thought. "It helps to learn from the experience of several drug candidates that did not succeed," says David Liu, a cell biologist at Scios Nova Inc. of Mountain View, California, which is developing bradykinin antagonists. "We're approaching sepsis with great caution," he says, "but not ignoring it."

–Richard Stone

Some Companies With Sepsis Drugs In Clinical Trials

Target	Company	Approach
Endotoxin/Lipid A	Ribi Immunochem	Prophylactic treatment with endotoxin
"	Xoma	Monoclonal antibody (mAb) to Lipid A
"	Chiron	mAb to Lipid A
Tumor necrosis factor (TNF)	Chiron/Miles	Mouse mAb to TNF
"	Centocor	mAb to TNF
"	Hoffmann-La Roche (with Genentech)	Fusion protein to bind TNF
Interleukin 1 (IL-1)	Synergen	Recombinant molecule that blocks IL-1 receptor
Bradykinin	Cortech	Bradykinin antagonists
Platelet Activity	British Biotechnology Group	Antagonist to platelet activating factor

The enemy within. Biotech companies target several steps in the immune cascade *(above)*, which is thrown off-kilter in patients with sepsis.