PHARMACOLOGY

War in the cells

Discoveries show how to help the immune system fight generalized infections

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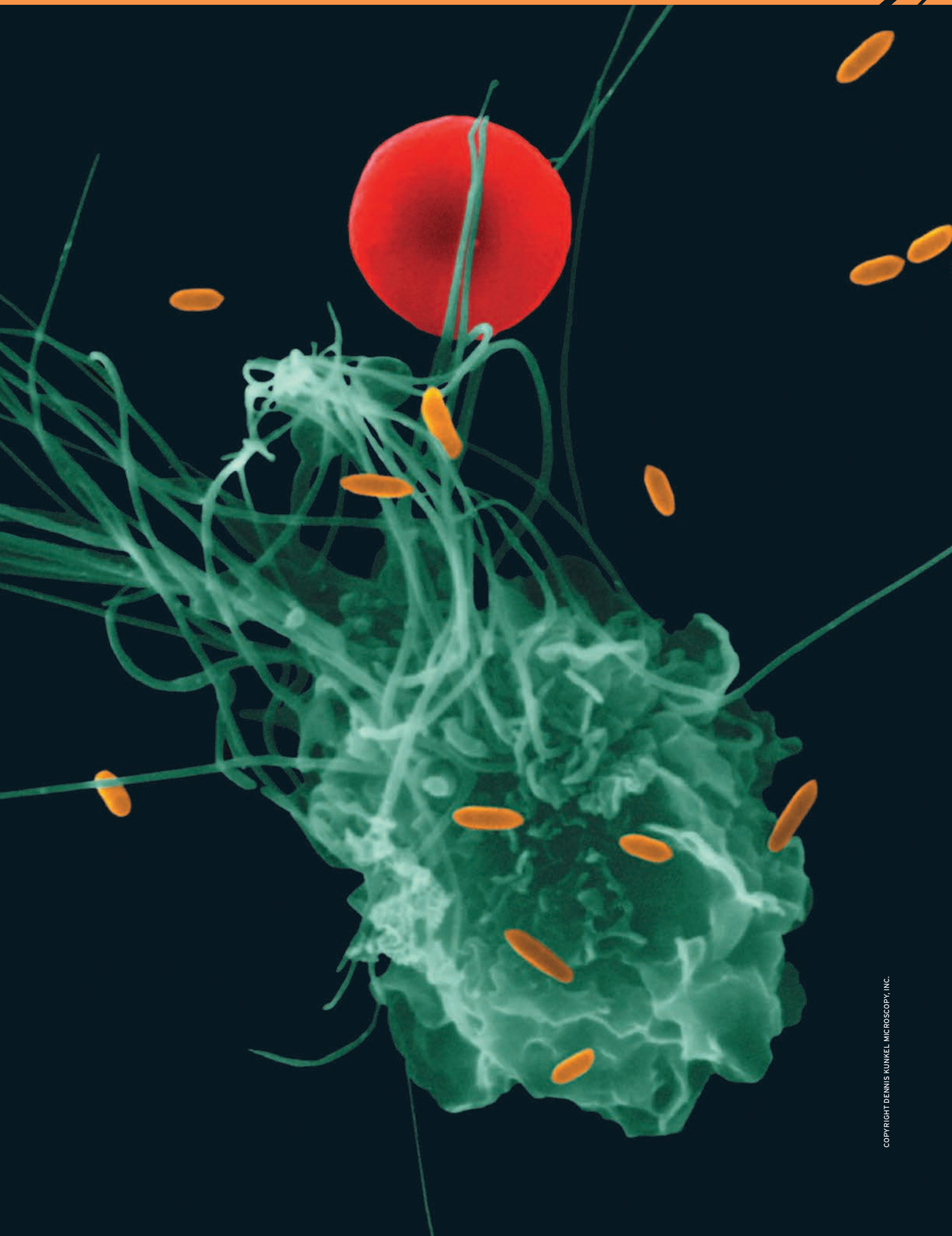
Be careful when turning the page. If you cut your finger on the edge of the paper bacteria will enter the wound and unleash a battle there. In these situations, the tissue defense cells, such as the macrophages, detect the invading bacteria, surround them and kill them. This process releases a series of substances around the cells that, like the bread crumbs in the story of *John and Mary*, indicate the path for leucocytes, defense cells that patrol the body in the blood stream. If all goes well, the infection will be controlled and will go unnoticed. But sometimes, because there are too many bacteria or because the immune system is compromised, this is not enough. The bacteria and inflammation spread through the body, causing generalized infection or sepsis. It is the second most common cause of death in Intensive Care Units (ICUs) in the United States, where more than 700,000 cases go on record every year – of which almost 30% lead to death. The team of pharmacologist Fernando de Queiróz Cunha, from the Medical School at the University of São Paulo in Ribeirão Preto (USP-RP), is revealing the battle of the immune system against sepsis and the direction to be taken in preparing medication.

Macrophave (*green*) captures bacteria in the lung



Cunha's work shows how the immune response works: in an inflammatory reaction the signaling substances advance to the closest blood vessel, attach themselves to the cell walls and send signals inside. The leucocytes then roll around inside the wall of the vessel until they find a breach through which they escape. Then they follow the chemical trail to the front line and join up with the macrophages to fight the bacteria, which they kill with substances like nitric oxide. The substances released in this process also cause an inflammatory reaction that attacks the tissue itself.

When the bacteria win the battle they spread throughout the body and generate sepsis, with the immune system in pursuit. In an extreme effort to contain the infection, the inflammation itself becomes generalized, causing blood pressure to drop and, in the end, multiple organ death. This is the situation currently known as sepsis (the word septicemia is no longer used much among experts). At least half of the people who succumb to this state die.

The great surprise for the international scientific community around ten years ago was to discover that the invading bacteria are not the most serious problem. The great damage happens because the inflammatory process, a valuable weapon in fighting bacteria, turns against the body itself – the same out-of-control situation that causes diseases like gout, arthritis and multiple sclerosis. It seemed obvious; all that was needed was to block the inflammation to contain sepsis. North American researchers tried, but without the inflammatory process, the fight against the infectious focus also stops and the bacteria spread unchecked.

To find an effective way of fighting sepsis, the group from Ribeirão Preto put together a three part research project. Physician and pharmacologist Sérgio Henrique Ferreira, project coordinator, is responsible for investigating the mechanisms that cause pain in an inflammatory process, while detailing the sepsis process and the migration of leucocytes to the focus of the infection is Cunha's task.

He discovered that the role of nitric oxide, which leucocytes use to kill bacteria, is central in septic shock. Within the blood vessels, this substance aids the defense mechanisms, because it induces the vascular muscles to relax; in doing so the greater volume of blood in the vessels carries more leucocytes to the focus of the infection. However, when there is sepsis, nitric oxide production goes uncontrolled and can be as much as a thousand times greater than normal, leading to a dramatic drop in blood pressure. Discovering this suggested a treatment: inhibit the patient's nitric oxide production. But what seemed like one more good idea gave rise to new problems. Without nitric oxide, neutrophyles lose their main microbicidic agent and are no longer able to fight the infection.

Cunha discovered something else: nitric oxide in excess also inhibits cell migration. "The leucocytes didn't stick to the vessel walls, didn't roll and didn't respond to the gradient of inflammation mediators," he says. In articles in the international journals *Shock*, *Blood* and *Critical Care Medicine* in 2006, Cunha's group detailed how this happens. The biochemical and protein paths, which lend cells movement similar to that of slugs, do not work in the presence of high levels of nitric oxide. In an article published in 2007 in the *American Journal of Respiratory and Critical Care Medicine*, the team from Ribeirão Preto also showed that nitric oxide inhibits the expression of receptors on the surface of neutrophyles,

Second-ranking cause of death in Intensive Care Units in the United States, sepsis is also a serious problem in Brazil.

In 2003, the Brazilian healthcare system earmarked more than R$ 17 billion for the treatment of 400 thousand sepsis patients, with unsatisfactory results, given that some 227 thousand of them died from severe sepsis.

which consequently lose their sensitivity to the inflammatory mediators. The immune system is therefore paralyzed, putting the patient's life at risk. This discovery suggested directions to Cunha's team. "If we were to re-establish the migration mechanisms, the infection would be controlled," he says.

That is what his team is trying to do now. They found that an essential substance in this biochemical chain is sulfhydric acid, also known as hydrogen sulfide (H_2S), the gas that makes rotten eggs smell bad. When its synthesis is inhibited within the leucocytes, cellular migration is halted; in returning H_2S to the cellular environment, the researchers saw that the defense cells started rolling again within the walls of the blood vessels. The strategy is new and the pharmacologist from Ribeirão Preto is now preparing an article for publication. For Cunha, the results encourage optimism. Perhaps now the understanding of sepsis is closer to allowing lives to be saved.

Until then, septic shock remains a public health problem with no solution. On the contrary, with the annual ageing of the population, a greater proportion of ICU patients suffer from sepsis. An article published in 2006 in *Endocrine, Metabolic & Immune Disorders – Drug Targets*, coordinated by Eliézer Silva, a doctor from the Intensive Therapy Center of the Albert Einstein Israelite Hospital in São Paulo and president of the Latin American Institute for Sepsis Studies (Ilas), compares the impact of sepsis in various countries and shows that for every one hundred people admitted to North American ICUs about ten go into septic shock.

In Brazil, Silva coordinated the study known as *Bases (epidemiological study of sepsis in Brazil)*, which evaluated 1,383 patients admitted to five Brazilian ICUs and that was published in 2004 in *Critical Care Medicine*. The study, one of the widest in the country, found that some 30% of these patients entered sepsis and developed septic shock. Intense medical care only managed to save half the patients with sepsis. The Brazilian Association of Intensive Therapy conducted another study, known as *Sepsis Brazil*, which examined more ICUs and achieved results similar to those of *Bases*.

According to data published by Ilas, in 2003 the Brazilian health system spent R$ 41 billion on intensive care. Of this amount, more than R$ 17 billion were earmarked for the 400,000 septic patients - with unsatisfactory results, since some 227,000 of them died as a result of serious sepsis, thereby taking to the grave an investment of almost R$ 10 billion.

To reduce these numbers, in 2005 Ilas joined the international campaign 'Surviving Sepsis'. With the aim of reducing death from septic shock by 25% by 2009, 48 countries are implementing international directives for the care of septic patients. To control and optimize the campaign results, participants send information to an international database. Brazil, with 50 institutions taking part in the program, is one of the countries that is contributing most data.

"The main difficulty is a cultural change," explains Eliézer Silva, who in 2006, through the Atheneu publishing house, published a manual for training professionals with regard to the new concept in which time is crucial. The new directives determine that when a patient with serious sepsis arrives for emergency treatment in a hospital, a blood sample must be collected immediately to identify the germ causing the infection. Then, within the first six hours, it is essential to give the patient antibiotics, large volumes of physiological serum and blood pressure stabilizing medication. Depending on the disease's progression during this time, another series of actions are required by the 24th hour of treatment: medicating with corticoids and activated C protein, controlling glycemia and, when the patient has difficulty breathing, supplying ventilation to maintain oxygen pressure at an appropriate level. The most recent data, not yet published, indicate that during the course of the campaign, death by sepsis dropped by almost 7% worldwide – at least as far as those that could be easily measured were concerned.

According to Fernando de Queiróz Cunha, discharging patients is not the same as breathing a sigh of relief. He showed from research with rats that has not been published yet that sepsis leaves the immune system weakened. The pharmacologist saw that 15 days after the septic crisis it is enough to spray bacteria close to an animal's snout (a situation not unlike talking to someone who has a cold) to cause death in guinea pigs. The work of Silva and Cunha makes clear the need for combining basic research, medical clinical studies and public policies to win the battle against sepsis. ■

THE PROJECT

Mediators involved in the genesis of pain, the migration of leucocytes and in sepsis

TYPE
Thematic Project

COORDINATOR
Sergio Henrique Ferreira - USP/Ribeirão Preto

INVESTMENT
R$ 2,277,550.31

Scientific articles

1.Rios-Santos, F. et al.Downregulation of CXCR2 on neutrophils in severe sepsis is mediated by inducible nitric oxide synthase-derived nitric oxide. **American Journal of Respiratory and Critical Care Medicine**. v. 175, p. 490-497, 2007

2.Torres-Dueñas, D. et al. Failure of neutrophil migration to infectious focus and cardiovascular changes on sepsis in rats: effects of the inhibition of nitric oxide production, removal of infectious focus, and antimicrobial treatment. **Shock**. v. 25, n. 3, p. 267-276, 2006