When the body destroys itself

We now have a better understanding of the molecular mechanisms of sepsis, a deadly inflammatory reaction caused by the immune system

Salvador Nogueira

Electron microscope image of *Capnocytophaga canimorsus*, one cause of sepsis

Imagine that in trying to expel an enemy from your land, an army employs so much force and savagery that it ends up destroying the very population it set out to defend. That's basically what happens when someone suffers from sepsis, an acute inflammatory reaction caused by the body's immune system as it reacts to an infection. Also known as septicemia (a term that has fallen into disuse), the disease is often fatal because it affects multiple organs and causes them to fail in a short period of time.

Scientists have slowly made sense of what happens to the body when it faces this exaggerated and desperate immune reaction. This is leading to the development of new treatments that have been effective in increasing patient survival. However, saving them once sepsis takes hold continues to be very difficult.

"Patients continue to die, but not in the early stages of the infection," says José Carlos Alves Filho of the University of São Paulo (USP) School of Medicine in Ribeirão Preto. "What we are seeing is that the later stage is much different than the early stage."

Alves Filho is a member of the group led by Fernando de Queiróz Cunha that for years has worked on decoding the mysteries of sepsis on a biomolecular scale. The team focuses on the mechanisms triggered in the cells and tissues that cause the body to have this deleterious reaction when it identifies the presence of a pathogen. And they discovered that sepsis, in a patient who survives long enough, leads to disruption of the immune system.

AN ICU ILLNESS

Not every infection causes sepsis. In fact, the immune system easily repels most bacteria that invade the body. Infections and widespread inflammation only occur when there is something wrong with the body's defense mechanism. That is why sepsis usually appears in someone who is already hospitalized, especially in hospital Intensive Care Units (ICUs).

"It's usually a secondary infection that leads to a patient's death," says Alves Filho. "First the person is hospitalized with an infection. That infection gets treated and then the patient gets a second infection while still in the hospital."

When the condition sets in, the results are disastrous. In the United States, for example, sepsis is the second leading cause of death in ICUs. There are over 700,000 cases of it every year, and nearly 30% of them lead to death. Worldwide, that number is 18 million cases each year. It is because of this that the subject has become a classic example of a new branch of research called "translational medicine."

It is the name given to an area of studies that arose out of the immediate need for physicians to turn to the laboratory in their attempt to get to the source of the problem and find new solutions. In this case, by unraveling the molecular enigma of sepsis, Queiróz Cunha and Alves Filho seek to identify effective pharmaceutical interventions that may cut short the process before it leads to death.

In the laboratory, sepsis is induced in rodents in an attempt to somehow mimic what takes place in hospitals. First the animals undergo intestinal perforation, which causes the primary infection. Peritonitis then appears, after which the researchers cause a secondary attack — pneumonia. The result is a rat stricken by sepsis. That is where the research begins to determine what happens and how the problem can be contained.

IMMUNE SYSTEM

With sepsis, we have known for over a decade that the main problem is not the infection itself, but rather the exaggerated immune response that not only fails to contain the invading bacteria, but spreads throughout the entire body, causing multiple organ failure. But intervention in this problem presents a dilemma much like Sophie's Choice: if physicians attack the immune system, the infection runs rampant. If they allow the system to act unimpeded, it ends up destroying the body.

What is needed then is a more refined strategy that is able to affect the system more subtly, without causing any type of disruption. And it

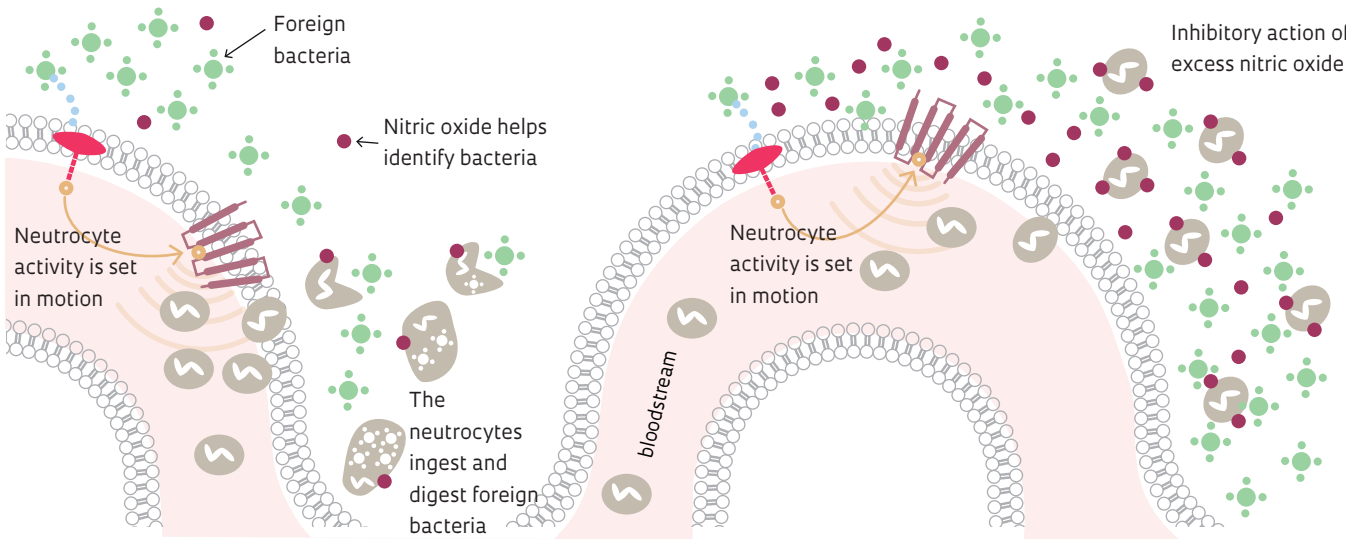
Cellular destruction

INFLAMMATORY RESPONSE

In a normal inflammatory scenario, neutrocytes, with the help of nitric acid, attack the foreign bacteria in the body

SEPSIS

Nitric acid production begins and inhibits the action of the neutrocytes, causing the infection to run rampant



INFOGRAPHICS TIAGO CIRILLO. SOURCE: FMRP/USP

is precisely knowledge of this that the Queiróz Cunha group seeks.

The group discovered, for example, that nitric oxide is an important component in the network of chemical reactions that lead to sepsis. Under normal circumstances, leucocytes (particularly one type of them – neutrophils) use nitric oxide to attack and destroy the bacteria. Nitric oxide also plays an important role in vascular relaxation. It allows blood flow to increase so that more defense cells are carried to the source of the infection.

However, according to prevailing medical wisdom, the difference between medicine and poison is all a matter of the dosage. With sepsis, nitric oxide production is at its highest. It can even be a thousand times higher than normal. This causes a sharp drop in blood pressure. And worse: any attempt to inhibit the patient's production of this substance eliminates the main weapon the leucocytes have against invading bacteria.

To make things even more dramatic, Queiróz Cunha and his colleagues discovered that excess amounts of nitric acid also inhibit migration of the defense cells, which helps explain how the condition becomes widespread. In 2006, the

Excess nitric oxide inhibits migration of defense cells, which helps explain how the condition spreads

details of this process were published in articles in the scientific journals *Shock*, *Blood,* and *Critical Care Medicine*. The following year, the group demonstrated why nitric oxide inhibits the action of neutrophils: they stop expressing certain receptors, which make them sensitive to inflammation. Cell migration is interrupted and it is as if the army defending the body unilaterally declares a ceasefire. In the meantime, the enemy continues to advance.

FLACCID HEART

An interesting development occurred when Queiróz Cunha's line of research met up with that of Marcos Rossi, a pathologist from the USP School of Medicine in Ribeirão Preto. Over the course of many years of performing autopsies on patients who had died of sepsis, Rossi had noticed that their hearts had undergone radical changes. "It was different, somewhat flaccid, which indicated that during its life, the heart wasn't functioning properly," says Rossi, who received the sum of R$850,000 through FAPESPS's Multi-user Equipment Program.

Rossi and Queiróz Cunha investigated the cause of this heart muscle devastation. Using mice as animal models for

the studies, the researchers determined that when affected by sepsis, there was a significant reduction in the number of proteins responsible for keeping the heart cells of the mice firmly attached to one another. As a result, the cells became detached.

Again the villain appeared to be excess nitric oxide. When released in excess during the inflammatory process, it damages the walls of the heart cells, making them more permeable to calcium. As a consequence, excess nitric oxide leads to cell death. The higher the number of cells that are affected, the more the heart's ability to pump blood is reduced.

The findings were published in the scientific journal *Shock* in 2010 and led to a promising strategy. Since several medications block calcium absorption and are used to control blood pressure and regulate heart rhythm, Rossi and Queiróz Cunha decided to try to use them in laboratory animals to see if the medications would protect the animals from sepsis. The results obtained in collaboration with researchers from the Albert Einstein College of Medicine in New York were very impressive.

The group conclusively demonstrated that there is a dramatic increase in the amount of calcium in the heart cells when the body is infected by sepsis. After just two minutes of going into septic shock, the body's calcium increases 60%. After 24 hours, it continues to increase by 20%. But the story is different when there is treatment.

"We showed that there is a marked improvement when the animals are treated with calcium blockers," says Rossi. While the control group (untreated mice) demonstrated a 90% mortality rate after 72 hours, in those that were treated, that rate fell to 50%. "Some might say, "even so, a lot of them died." But the point is that we improved the survival rate five times over."

The main problem is that sepsis is a widespread assault. It drastically affects the heart as well as other organs. The researchers developed a way to protect the heart muscle, but even so, in many cases, the animals continued to die – this time because other organs failed.

GOING BACK TO THE HOSPITAL

For Rossi, much remains to be learned about the mechanisms of sepsis. Nevertheless, he thinks the specific work with calcium blockers is ready for clinical trials. Once these drugs are approved for limited use, it will significantly shorten the test time involved in converting it into a useful tool to protect the heart during a bout with sepsis – which on its own, would already significantly reduce mortality rates, at least in the early stages of the illness.

Despite this potential, no group that specializes in research with humans has sought him out to put the strategy into practice. "There's a lot of talk about translational medicine, but it is not in practice. People do things in the laboratory, but there is no one to take it from there back to the hospital. And in Brazil, people are even more reluctant," he criticizes.

While they wait for the transfer to happen, Rossi and Queiróz Cunha continue to work on uncovering all the molecular mechanisms involved in this conflict that pits the body against itself. And their studies continue to present potential targets for medical intervention in the great challenge that lies in defeating sepsis. ■

PROJECTS

1. *Mediators involved in the genesis of pain and the migration of leucocytes in sepsis* – No. 2007/51247-5 (2007-2012)
2. *Sepsis and septic shock: functional and morphological alterations of the heart: experimental study in mice* – No. 2004/14578-5 (2005-2007)
3. *In vitro assessment of dystrophin expression in cardiomyocytes subjected to different stimuli* – No. 2009/53544-2 (2010-2012)

GRANT MECHANISMS
1. and 2. Thematic project
3. –Regular Line of Research Project Award

COORDINATORS
1. and 2. Sergio Henrique Ferreira – (FMRP-USP)
3. Marcos Antonio Rossi – (FMRP-USP)

INVESTMENT
1. R$2,303.227.35
2. R$153,565.78
3. R$310,920.30

SCIENTIFIC ARTICLES

1. ROSSI, M. A. *et al.* Myocardial structural changes in long-term human sepsis/septic shock may be responsible for cardiac dysfunction. **Shock**. v. 27, n. 1, p. 1-18, 2007.

2. CELES, M. R. *et al.* Disruption of sarcolemmal dystrophin and beta-dystroglycan may be a potential mechanism for myocardial dysfunction in severe sepsis. **Laboratory Investigation**. v. 90, p. 531-42, 2010.

3. CELES, M. R. *et al.* Reduction of gap and adherens junction proteins and intercalated disc structural remodeling in the hearts of mice submitted to severe cecal ligation and puncture sepsis. **Critical Care Medicine**. v. 9, p. 2176-85, 2007.

4. CELES, M. R. *et al.* Increased sarcolemmal permeability as an early event in experimental septic cardiomyopathy: a potential role for oxidative damage to lipids and proteins. **Shock**. v. 33, n. 3, p. 322-31, 2010.

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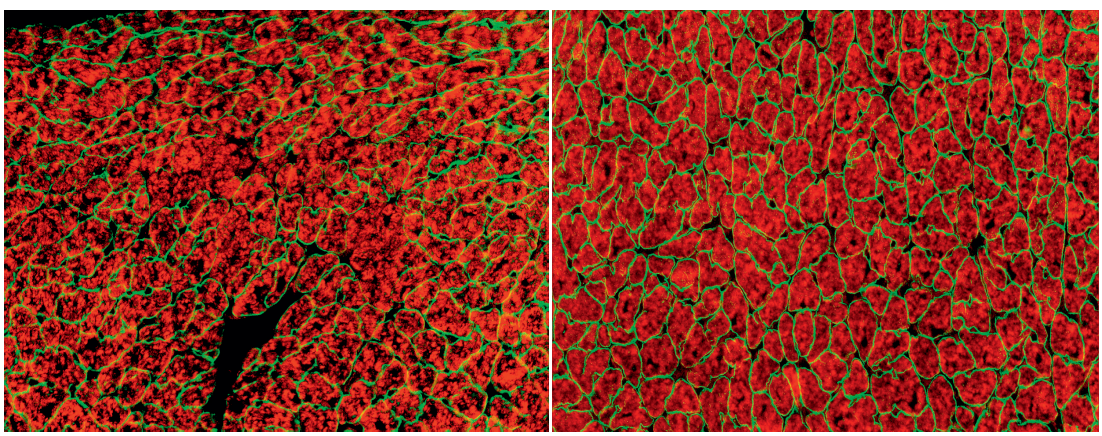

PHOTOS MARCOS ROSSI/FMRP-USP

Sepsis alters the structure of the dystrophin protein (in green, on left) and impairs the action of the heart cells (normal condition, on right)