

[IMMUNOLOGY]


Out of **control**

Inflammation
unleashed by
sepsis damages
the heart

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Defending the organism from itself when it launches a desperate attack against its own cells is the main challenge facing doctors in cases of sepsis, a generalized infection caused by bacteria or a virus, accompanied by aggressive inflammation that attacks the organs it should protect. Assessing the health of patients with sepsis, a problem that every year affects 18 million people worldwide, doctors in Brazil and from other countries have observed that the risk of dying increases a lot when the most damaged organ is the heart: the death rate reaches 80% if the heart muscle is affected and starts pumping oxygen-rich blood less efficiently to the rest of the body, compared to 20% when there is no heart damage.

Now researchers at the University of São Paulo (USP) in Ribeirão Preto have gone a step further. Analyzing the hearts of people and animals that died from sepsis, the team, coordinated by pathologist Marcos Rossi and by pharmacologist Fernando Cunha, has characterized the type of damage that causes sepsis-related inflammation in cardiac cells. More importantly, it also found a promising way to protect the heart and thus gain time for the body to regain control of the situation.

The main advance of the group from Ribeirão Preto was to see what happens with the heart cells on a molecular scale. In studies with laboratory animals the researchers discovered that molecules of nitric oxide released in the inflammation damaged cell walls making them more permeable to calcium. The consequence of this alteration is an overdose of this particular chemical element that leads to cell death – if the number of cells affected is very large, it reduces the capacity of the heart to pump blood. Published in March 2010 in the scientific journal *Shock*, this finding is significant because it suggests ways of slowing down the process of wear and tear in the heart. There are drugs on the market that block the absorption of calcium and that are used to control blood pressure and to regulate the heartbeat.

Currently, Cunha and Rossi's group, in partnership with researchers from the Albert Einstein College of Medicine in New York, are evaluating if these drugs really help keep the heart functioning when administered during sepsis. The study is still ongoing, but preliminary results are fairly expressive. In one of the experiments the researchers administered compounds that prevent the absorption of calcium – the so-called calcium channel blockers – to mice that had suffered a perforation of the intestines and had developed a generalized infection. Then, they compared what happened with a group of animals with untreated sepsis and with a group of healthy rodents.



THE PROJECTS

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

TYPE

- 1 and 2. Thematic Project
3. Regular Aid for Research Project

COORDINATORS

- 1 and 2. Sergio Henrique Ferreira - USP/RP
3. Marcos Antonio Rossi - USP/RP

INVESTMENT

- R\$ 2,303,227.35
R\$ 153,565.78
R\$ 310,920.30

The calcium channel blockers provided some degree of survival to the sick mice. Without the medication most of the animals with sepsis died in less than 24 hours. When treated, however, all survived the first day. “The death rate of animals with sepsis that received the calcium blocker was similar to that of mice in the control group that had no infection,” explains Rossi. “We’re enthusiastic about the results.”

Many more tests are still needed – and possibly years of work – to prove whether this strategy is efficient and can be adopted safely on a daily basis in hospitals. However, one fact makes the researchers optimistic: it will be simpler to carry out tests on humans, since calcium channel blockers are already used to treat heart problems. Rossi remembers, however, that it is premature to suppose that everything is going to work out all right, because the circumstances to which the animals were subjected are quite different from those involving patients in hospitals.

As a pathologist, Rossi performed many autopsies on patients who had died from sepsis and found that almost always their hearts had undergone radical changes. “The heart of a patient with sepsis was different, somewhat flaccid, indicating that during its life it had had functioning problems,” he says. Analysis of material obtained from autopsies in fact indicated morphological changes in the cardiac muscle. Presented in *Shock* in 2007, these changes were like a picture of the final moment.

In order to know how sepsis-associated heart damage begins and evolves the researchers had to resort to an experimental model of the problem – they chose to work with mice, because the organism of these rodents functions in a similar way to that of humans. By means of an incision in the animal’s intestine bacteria from the digestive tract reach the thoracic cavity and cause a generalized infection.

Right from the start the researchers noted an important change in the structure of the heart of the animals that developed sepsis: there was a significant reduction in the number of proteins responsible for keeping the heart cells strongly united. As a result, these cells, known as cardiomyocytes, separated from each other, Rossi observed when analyzing the tissue under an electronic microscope. It was as if, at the cell level, the heart muscle had been ‘dismantled’.

Even if this transformation, which was described in 2007 in *Critical Care Medicine*, were to occur at the microscopic level, the ‘dismantling’ produced easily observable consequences. For the heart to beat regularly, its cells need to be firmly attached to one another in such a way that they contract or relax in harmony. With the cells disconnected the heart rhythm became irregular and the heart quickly stopped.

More sophisticated chemical analyses, using a technique (immune-fluorescence) that makes certain proteins shine when present in a sample, reinforced the suspicion that cardiac re-



structuring occurred at the molecular level, but not inside cells. The problem was outside, in the so-called extracellular environment. The group noted that a protein structure, the dystrophin-glycoprotein complex (DGC), which serves as support and shapes the cells, seemed to dissolve in the hearts of animal victims of sepsis, the researchers from Ribeirão Preto revealed in an article published in *Laboratory Investigation* in April 2010.

If this heart damage is indeed caused by the inflammation associated with sepsis, the solution for increasing the survival rate of those who develop the most serious forms might be in controlling the inflammation and the damage caused by it. According to the researchers from Ribeirão Preto, this would be an important transformation in the way of dealing with the problem, since attempts are generally only made to fight the infectious agents with antibiotics and antiviral drugs. “The changes identified are therapeutic targets, whose modulation may reduce morbidity and mortality in sepsis,” says Rossi.

They are not the only ones who think so. At the University of Utah, in the United States, the group led by cardiologist Dean Li, of which Brazilian physician, Fernando Augusto Bozza, from the Evandro Chagas Institute of Clinical Research, in Rio de Janeiro forms part, tried to control inflammatory reactions resulting from sepsis or bird flu in an unusual way. Researchers gave the mice a compound that prevented the chemical communicators that feed the inflammation from leaving the bloodstream and reaching the tissue. In this way they managed to reduce the level of damage to the organism of the rodents, according to an article published in *Science Translational Medicine* on March 17. “By blocking the harmful effects of the inflammation in the host and stabilizing the blood vessels, we identified a totally different strategy for treating these in-

Inflammatory molecules make the membrane of heart cells more permeable to calcium, leading to an overdose and cell death

fections,” said Li. “In essence, we show that instead of attacking the pathogen, we can target the host to help it fight the infection.”

Adequate control of sepsis, however, should demand more of an action strategy. In a recent study made in partnership with researchers from the University of Glasgow, in Scotland, pharmacologist José Carlos Alves Filho, from Cunha’s team, gave mice with sepsis a protein, which is naturally produced by cells in the defense system, which acts as a chemical communicator of anti-inflammatory action: interleukin 33 or IL-33. Besides reducing inflammation in the organism without eliminating it in the original center of infection, this protein stimulated the migration of a specific type of defense cell – neutrophils – which eliminate bacteria efficiently.

The results of this experimental therapy were clear. Only 20% of the rodents treated with IL-33 died of sepsis, while the mortality rate in the group that received an innocuous compound

was 80%. In the article in which they presented these data in *Nature Medicine* on May 16, the researchers suggest that the effect the IL-33 produced in mice is likely to be also observed in humans, since neutrophils are less active in people who develop more serious cases of sepsis.

Less than a month before, another member of Cunha and de Rossi’s team, pharmacologist Fernando Spiller, had shown that the use of hydrogen sulfide, or hydrosulfuric acid (H₂S), the gas responsible for the stench of rotten eggs, induces a migration of neutrophils and another defense cell group, the leucytes, to the initial infection area (see Pesquisa FAPESP nº 146). This cellular reinforcement eliminated the bacteria and reduced the mortality rate among the mice receiving the compound to 13%, compared to nearly 80% among those not treated, according to an article published in *American Journal of Respiratory and Critical Care Medicine*.

Despite being encouraging these advances only represent the initial step on a long journey towards improving the control of sepsis, a public health problem that is especially serious in developing countries, where resources are scarcer. A survey done years ago by the Latin American Institute for Sepsis Studies showed that of the R\$ 41 billion spent in 2003 on intensive therapy by the Brazilian health system, more than R\$ 17 billion was used to treat 400,000 patients with sepsis, of which 227,000 died. ■

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 (1), p. 1-18. Jan. 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. Feb. 2010.