Discovered: The mechanism that makes infants more likely than adults to die from sepsis

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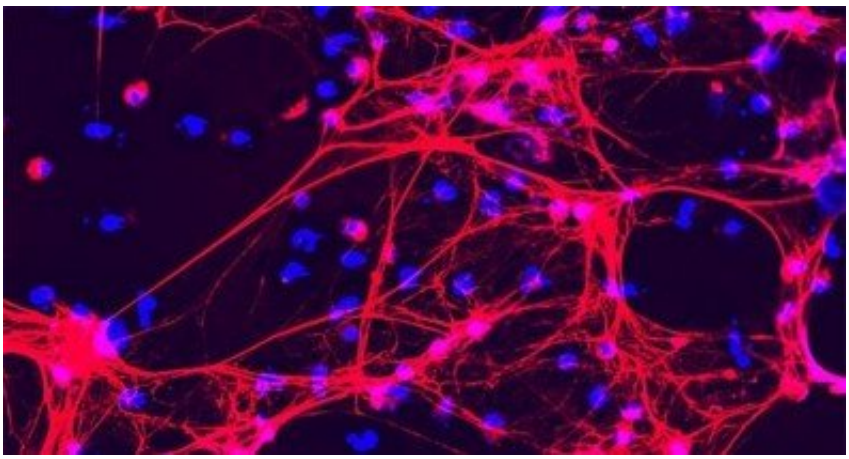

Scientists at the Center for Research on Inflammatory Diseases (CRID) show why pediatric patients with sepsis suffer from more inflammation and organ injury than adults. New treatment strategies may be tested. Credit: Mariana J. Kaplan / NIAMS Systemic Autoimmunity Branch

An immune mechanism that makes babies more likely than adults to die from sepsis has been identified by scientists affiliated with the Center for Research on Inflammatory Diseases (CRID in Ribeirão Preto, São Paulo State (Brazil). The study is published) in *Critical Care*.

The scientists are planning to test new therapeutic approaches based on the discovery. "We're designing a clinical trial with drugs that have been

approved for human use and are known to induce this immune mechanism.The goal is to improve the survival rate for infants with sepsis," said Fernando de Queiroz Cunha, CRID's principal investigator. CRID is one of the Research, Innovation and Dissemination Centers (RIDCs funded by São Paulo Research Foundation—FAPESP.

Sepsis (sometimes referred to as blood poisoning) is systemic inflammation usually triggered by a localized bacterial infection that spins out of control. The body's immune response to combat the pathogen ends up damaging multiple organs and tissues.

Symptoms include fever or low temperature, difficulty breathing, low blood pressure, a fast heart rate, and an abnormally high or low white blood cell count. The condition may remain active even after the initial threat has been eliminated. Its most severe form can lead to lesions that impair the function of vital organs, septic shock and death.

"In any experimental animal model of sepsis, all the parameters used to measure the severity of the condition are higher in infants. There's more systemic inflammatory response, more organ impairment, and higher mortality," said Cunha, who is a Full Professor in the Department of Pharmacology at the University of São Paulo's Ribeirão Preto Medical School (FMRP-USP).

In humans, it is more difficult to compare infant and adult mortality rates, he explained, because, before contracting sepsis, the adult patient may have been weakened by diseases such as diabetes, cancer, heart failure or hypertension (high blood pressure). "Most adults who die as a result of septic shock already had serious health problems," Cunha told.

Given their knowledge that organ injury is more severe in young individuals, both human and murine, the group decided to determine exactly what substances are produced by the immune system during

sepsis. Their hypothesis was that defense cells in infants must produce more oxidizing substances, such as oxygen and nitrogen free radicals. What they found, however, was the opposite.

"We took a long time to understand why infants have more tissue injury if they produce smaller amounts of free radicals. Finally, we decided to investigate NETs [neutrophil extracellular traps]," Cunha said.

Neutrophils are white blood cells that form the front line of the immune system, phagocytosing (killing) bacteria, fungi and viruses. NETs are structures composed of DNA and granular proteins that rapidly trap and kill pathogens.

"This immune mechanism was first described about ten years ago. In some situations, for poorly understood reasons, the immune system activates an enzyme called PAD-4, which increases the permeability of the neutrophil nucleus. When this happens, the genetic material in the nucleus decondenses and forms networks, which are released by the cell into the extracellular medium to trap and kill bacteria," Cunha said.

NETs are typically activated by bacterial infections, he added, as well as some viruses, including *chikungunya*, the arbovirus that causes the most tissue injury. The mechanism also occurs in some autoimmune disorders. "The main problem is that NETs aren't just toxic for pathogens: they also damage human cells. In fact, they do more damage than oxygen and nitrogen free radicals."

Tests involving pediatric patients were conducted in collaboration with a research group led by Professor Ana Paula Carlotti, attached to the ICU at FMRP-USP's teaching and general hospital (Hospital das Clínicas). Laboratory analysis showed that neutrophils from infants produced 40 percent more NETs than those taken from adults, in the case of humans. The difference was 60 percent in mice. The group then set out to use

experimental models to understand how this immune mechanism works in sepsis.

Traps deactivated

The experiments with mice involved a group of two-week-old infants and a group of healthy young adults. Both received an intraperitoneal injection of intestinal bacteria and developed sepsis.

"A dose of bacteria sufficient to kill 100 percent of infants killed only 50 percent of the adults. That's a significant difference. Moreover, in the days following the injection, the infant mice displayed higher levels of bacteremia [bacteria in the bloodstream] and of biochemical markers indicating organ injury," Cunha said.

When NETs were broken down with recombinant human DNase (a drug used to treat cystic fibrosis), the survival rate jumped from 0 to 50 percent in the infant group. In the adult group, the proportion of mice that survived sepsis rose from 50 percent to 60 percent.

"The difference between the groups when treated with DNase was small, clearly showing that greater infant susceptibility is associated with higher levels of NETs," Cunha said.

In another experiment, the group replaced DNase with a compound designed to inhibit PAD-4, the enzyme that triggers the activation of NETs. In this case, the survival rate for the infant group was 40 percent.

"It was somewhat less effective than DNase because it's not actually a specific PAD-4 inhibitor. One of our goals for future research is the development of a specific drug to inhibit PAD-4," Cunha said.

The group analyzed the expression of the PAD-4 gene, which encodes

the PAD-4 enzyme, in neutrophils from patients and from mice. In both cases, PAD-4 expression was higher in infants with sepsis than in adults with the same condition. The reasons are unknown and are currently being sought by David Fernando Colón Morelo, the first author of the article. Cunha is Morelo's Ph.D. supervisor.

Morelo has a doctoral scholarship from FAPESP and is now doing a research internship at Bonn University in Germany.

"We're also studying the role of NETs in other diseases involving organ injury, such as rheumatoid arthritis and lupus," Cunha said.

More information: David F. Colón et al, Neutrophil extracellular traps (NETs) exacerbate severity of infant sepsis, *Critical Care* (2019). DOI: 10.1186/s13054-019-2407-8

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