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Histone Molecules Drive Organ Failure During Sepsis

DNA is tightly packed in the nucleus of every cell. DNA wraps around special proteins called histones, which form loops of DNA called nucleosomes. These nucleosomes coil and stack together to form fibers called chromatin. Chromatin in turn forms larger loops and coils to form chromosomes.

When the immune system responds to an infection by going into overdrive, small blood clots can form, blocking blood flow to vital organs. The condition, called sepsis, kills about 215,000 people in the United States annually. New research shows that histones — the protein spools that help keep DNA tightly coiled — that escape from cells at the onset of sepsis contribute directly to organ failure.

The work demonstrates that histones aren't just a byproduct of sepsis, they're a ringleader in its development. The new studies point to histones as a new and potentially inviting drug target for tamping down the runaway immune reaction. In animal studies, Howard Hughes Medical Institute investigator Charles Esmon and colleagues showed that blocking histones with an antibody or enzyme prevents their destructive effects. Their findings are published online in the October 25, 2009, issue of the journal *Nature Medicine*.

“People had seen histones out in the circulation before and they'd say, ‘So what?’ But it wasn't so what. These guys are really bad players,” said Esmon, whose lab is at the Oklahoma Medical Research Foundation in Oklahoma City.

Histones are part of the body's rapid response to infectious disease, Esmon explained -- a stopgap measure until the immune system can kick into gear. “But they're not very specific, so you get a lot of injury.” The extent of that injury, he says, was far more dramatic than the team anticipated.

“I didn't expect to see this at all when postdoc Jun Xu started this project,” Esmon said. “Histones are not just bystanders; they're not just small contributors to a late-stage process. They're major.”

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Esmon's earlier research on an enzyme called activated Protein C (APC) led to the creation of the only drug now used against sepsis. That drug, Xigris, manufactured by Eli Lilly, is a form of the body's own APC enzyme. It reduces the risk of death from sepsis by about 20 percent. Xigris is useful only for severe sepsis, once organs have begun to fail, and bleeding problems associated with Xigris limit its use when sepsis arises after trauma or surgery. Sepsis still kills nearly 30 percent of its human victims in the United States.

Esmon's team set out to find another approach to treating sepsis by looking more closely at how APC halted its progress. Xu induced human macrophage cells to produce a sepsis-like response, and then stopped the response with APC. Amid the rescued cells he discovered fragments of cleaved histones. Esmon says the finding was a complete surprise.

In previous studies, Esmon's team used baboons to study APC's effects on sepsis. The animals in those experiments had been rescued with APC after receiving lethal levels of the bacteria *Escherichia coli* to produce septic shock. When the researchers examined blood samples collected during those experiments, they confirmed what Xu's *in vitro* test had suggested—that histones had a role in septic shock, and their cleavage halted the disease.

In further studies in Esmon's lab, researchers discovered that histones alone—in the absence of injury or invading bacteria—could produce a sepsis reaction in human endothelial cells and in mice. Although the histones can help ward off infection, Esmon said, their experiments demonstrate that the consequences of their release can be severe.

"This is the fast response, before you have time to start making antibodies. Unless you can stop pathogens from multiplying, you're not going to be around to make antibodies," he explained. But this early response can be like slapping mosquitoes with a hammer. You eliminate the bug, but the collateral damage is troublesome. Histones rush out of the nucleus and into the blood stream to capture bad guys in nets of DNA, but the histones themselves are toxic.

The histones rough up the lining of the blood vessels, the endothelium, Esmon said. That injury releases more histones, and blood vessels grow leaky. Clots then form in the tiny air sacs of the lung, and kidney function breaks down. And with each bit of tissue damage, each organ failure, more histones escape in a cascade of events that grows more damaging, and more

difficult to control, at every stage. Ultimately, patients slip into multiple organ failure and finally death.

That disease progression was halted in both the cultured cells and the mice when the team blocked the histones – either with an antibody or with APC. Both treatments improved survival among animals undergoing sepsis.

Importantly, a drug that could block histones has promise for more than septic shock patients, Esmon said. “We’ll do additional experiments with other animal models to test the extent to which this can be extrapolated to other diseases; how valid it is in other animals, and ultimately we’re hoping to go into the clinic.”

He plans to look for histones in the blood of patients with autoimmune disease such as lupus or type 1 diabetes, as well as any disease with major complications in blood vessels, including myocardial infarction and reperfusion injuries, and even certain types of lung disease.

“In those diseases, in those patients with high levels of circulating histones, we want to target those to see if we can make a difference,” Esmon said. “I think this has potential to be useful in broad range of diseases.”