Healing badly damaged lungs: Distinct set of white blood cells found to set the pace of wound repair

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After more than 50 experiments in mice, medical scientists at Johns Hopkins have mapped out the basic steps taken by a particular set of white blood cells in setting the pace of recovery after serious lung injury.

The white blood cells are called regulatory T cells, or Tregs for short, and their best known function is to keep the body's immune system from attacking its own healthy tissues.

"Our study results are the critical first leads in finding treatments for a clinical condition that until now has had none, despite its high mortality," says study senior investigator and pulmonologist Landon King, M.D. "When a patient develops acute lung injury, we want the critical care medicine team to be able to do more than just stabilize the patient on a ventilator," adds King, director of pulmonary and critical care medicine at the Johns Hopkins University School of Medicine.

King says the study opens the door to a new field in research and development of drugs that either speed up the post-injury activation of Tregs, or supplement levels of Tregs in people who may be relatively lymphocyte deficient from either lung disease or chemotherapy. Lymphocyte is the technical term for a type of white blood cell.

Some 200,000 Americans suffer some form of sudden, acute lung injury (ALI) each year, in which inflammation has spread across both lungs,

making breathing difficult and starving the body of much-needed oxygen. Among them are people with severe acute respiratory distress syndrome caused by infection, the most severe form of ALI. Also included are burn victims, people with chest injuries from car accidents, and cancer patients who have had adverse reactions to donated platelets from blood transfusion. Almost all people with ALI require breathing assistance from mechanical ventilators, and nearly 75,000 die each year.

The team of Johns Hopkins lung experts, whose study results are set to be published in the *Journal of Clinical Investigation* online Sept. 21, says their three-year investigation is believed to be the first to distinguish the role of the immune system in wound repair in the lung from its role immediately following injury and the inflammation that follows. They add that the study is also the first lab and clinical analysis to show how the body's built-in system of naturally occurring Tregs can be sped up or slowed down, either aiding or hindering healing in severely damaged lung tissue.

Spurred by the lack of treatment options, King and his team started to track and map out the biological steps involved in ALI recovery with an experiment in mice that were already serving as a lab model for lung injury. As part of the lab model, mice inhale a bacterial substance well known to critically injure both lungs within 24 hours, with inflammation, on average, peaking after four days. In mice that survive, the average recovery time is 10 days.

Researchers had long assumed that an initial spike in blood lymphocyte levels deep inside lung tissue cavities, which occurs in mice as well as in humans, was the immune system's first response to inflammation from injury. But the Hopkins scientists were surprised to find a higher death rate in mice that had been genetically modified to be lymphocyte deficient, at 40 percent, while the death rate in mice with lymphocytes was just 10 percent.

After determining that lymphocytes were key to mice recovering from lung injury, the team set out to sort out the various roles, if any, played by specific lymphocytes, by injecting injured mice with different types and combinations of the white blood cells, such as CD4 and CD8. Only transfusions of spleen cells rich in CD4 type T lymphocytes restored recovery time to normal.

Further experiments led by Johns Hopkins pulmonologist Franco D'Alessio, M.D., showed that blood levels of one subset of CD4 T lymphocytes, technically known as CD4+ CD25+ Foxp3+, or regulatory T cells, jumped proportionally in the lung spaces from the first day of injury, before peaking at day seven and remaining high throughout recovery at day 10.

This proved to D'Alessio and colleagues that the spike was not just a response to injury but also part of the lung's immediate and natural process of wound repair. When researchers removed Tregs from the damaged lungs in mice, leaving all other lymphocytes behind, lung resolution was again slowed by half.

"T cells and the body's active immune system play a crucial role in recovery from acute lung injury," says D'Alessio, an instructor based at Johns Hopkins Bayview Medical Center, "It is by no means a passive process as previously thought."

To test the prospects of Tregs as a potential therapy, the scientific team doubled bacterial toxin exposures, increasing death rates to 50 percent in untreated mice. But mice injected with doses of a million Tregs within an hour of exposure had just a 10 percent death rate, with many showing signs of accelerated recovery after just six days. Similar doses of Tregs provided days after exposure also showed lowered deaths rates in mice with ALI.

Additional lab testing showed that lung-tissue levels of other immune system cells involved in inflammation, notably neutrophils and macrophages, also dropped in response to the influx of Tregs, providing further evidence for Tregs controlled the switch in the lung tissue, "from an environment of inflammation to one of recovery."

"Our study should spark lung experts here and elsewhere to shift their research focus from nearly universal interest in the onset of acute lung injury to new mechanisms underlying resolution of lung injury," says King, an associate professor at Johns Hopkins, where he also holds the David Marine Professorship in Medicine.

Initial results from lung tissue extracts from two people with an ALI, also reported in the latest study, showed that within 48 hours from injury, Treg levels were 10 times normal.

King says his team's next steps are to identify the process by which Tregs orchestrate the transition of the mouse immune response from injury to repair, isolating and analyzing Tregs over the whole recovery period in the lungs, with the goal of identifying further biological drug targets for speeding up recovery. He notes that experiments elsewhere are already under way using Tregs in treating people with leukemia to prevent rejection of bone marrow transplants.

Source: Johns Hopkins Medical Institutions