Microbiome implicated in sickle cell disease—but antibiotics can counter its effects

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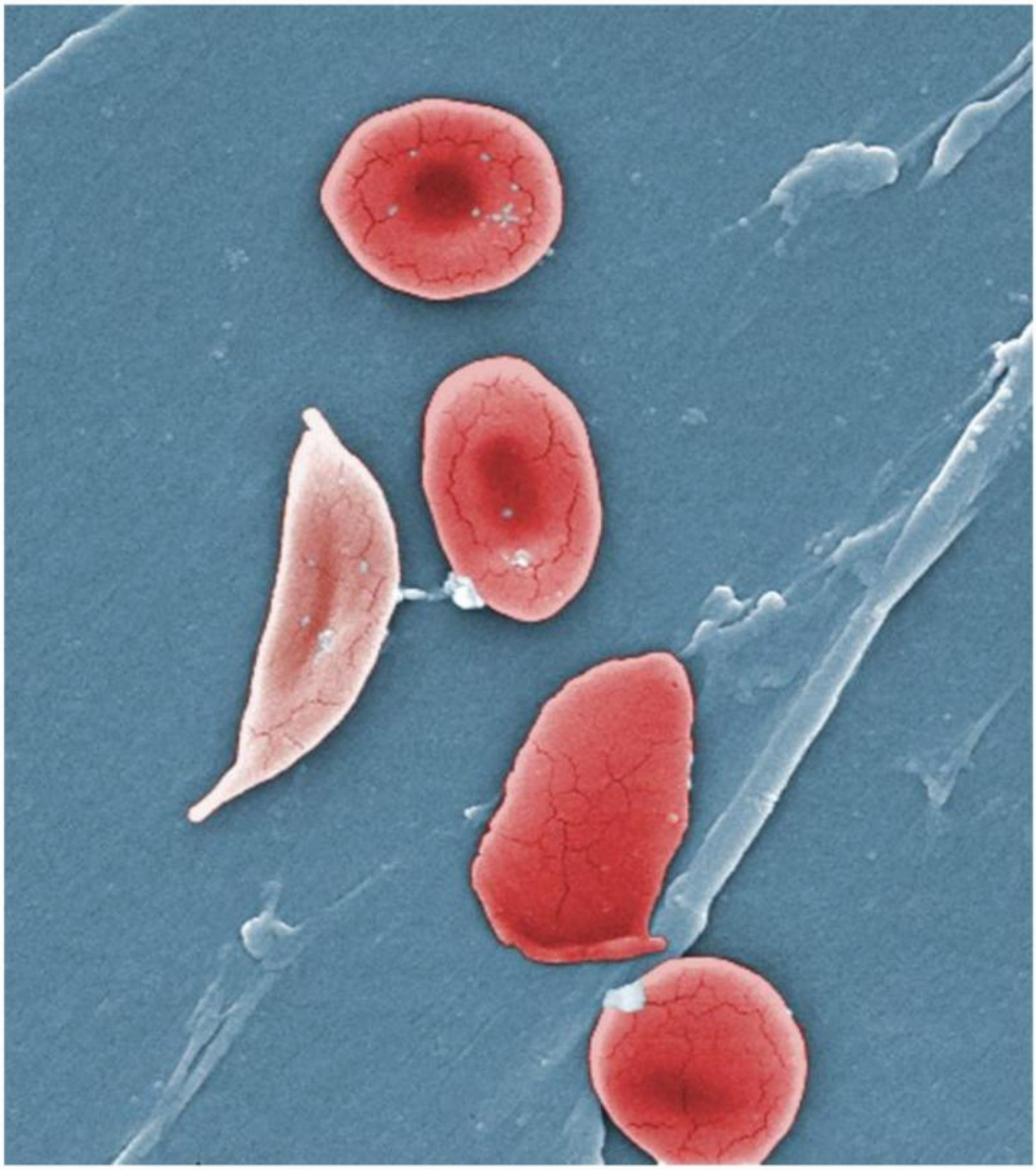

Normal blood cells next to a sickle-blood cell, colored scanning electron microscope image. Credit: Wikipedia/Illustration from Anatomy & Physiology

New research on sickle cell disease (SCD) has found that using antibiotics to deplete the body's microbiome may prevent acute sickle cell crisis and could offer the first effective strategy for warding off the disease's long-term complications, such as organ failure. The study, conducted by scientists at Albert Einstein College of Medicine and Montefiore Health System, could also lead to better treatment for other inflammatory blood-vessel disorders including septic shock. The findings were published online today in *Nature*.

SCD affects approximately 100,000 Americans and occurs in about 1 in 500 Black or African-American births. The disease affects millions of people throughout the world, particularly those with ancestors from sub-Saharan Africa and Spanish-speaking regions in the Western Hemisphere (South America, the Caribbean, and Central America.)

People with the disease have an inherited gene mutation that leads to abnormal hemoglobin, the red-cell protein that carries oxygen to the body's tissues. Red cells with abnormal hemoglobin take on a sickle shape and become less flexible. The sickled red cells tend to clog small vessels—impeding blood flow and preventing oxygen from reaching tissues. This can result in sudden attacks of severe pain called sickle cell crisis, or vaso-occlusive crisis, which often require hospitalization.

Over many years, the poor oxygen delivery due to SCD can damage organs including the spleen, liver and kidneys. On average, Americans with the disease can expect to live only into their mid-40s.

The Einstein study was led by Paul Frenette, M.D., professor of medicine and of cell biology and chair and director of Einstein's Gottesman Institute for Stem Cell and Regenerative Medicine Research. Dr. Frenette reported in 2002 that SCD vessel blockages occur when sickled red cells bind to white cells called neutrophils that have adhered to the vessel walls. Neutrophils are the most common type of white cells

in the blood and protect against disease-causing microbes.

"This earlier work indicated that not all neutrophils are the same," said Dr. Frenette. "Some appear to be inert while others appear overly active in promoting inflammation—which is useful for attacking microbes but causes neutrophils to capture sickled red cells inside vessels. So in the current study, we investigated whether the age of the neutrophils might be influencing whether they become active and pro-inflammatory."

Certain surface proteins reveal whether neutrophils are resting or have become active; different cell-surface proteins indicate whether neutrophils are young or old. After transfusing whole blood into mice and then analyzing young neutrophils (harvested 10 minutes post-transfusion) and aged neutrophils (harvested six hours post-transfusion), Dr. Frenette and colleagues found that neutrophils became more active as they age in the circulation—suggesting they receive some kind of external signals telling them to age.

The researchers carried out experiments that traced these "aging" signals to the body's microbiome. They found that the microbiome produces chemicals that cross the intestinal barrier and enter the bloodstream, where they generate the aged, overly active subset of neutrophils that contributes to SCD. "Since the body's microbiota seem to "educate" neutrophils to age," said Dr. Frenette, "we realized that purging those microbes through use of antibiotics might help against SCD."

To find out, Dr. Frenette's team carried out studies on a mouse model of SCD. They found that SCD mice possessed five times as many aged neutrophils as healthy control mice. When the researchers depleted the microbiota of SCD mice using antibiotics, they observed a striking reduction in neutrophils but not of other white cells such as monocytes, T cells and B cells. Moreover, giving antibiotics to SCD mice appeared to prevent sickle cell crisis: interactions between neutrophils and red

cells were markedly reduced in microbiota-depleted SCD mice, resulting in improved local blood flow and greatly improved survival of these mice.

"What was most surprising and exciting to us was the effect of antibiotics on chronic tissue damage," said Dr. Frenette. "We found that the spleen enlargement of SCD mice was significantly reduced in the microbiota-depleted animals, and liver analysis revealed major reductions in liver damage including inflammation, scarring and tissue death. This is the first time that anything has been found to have an impact on the organ damage that can be so devastating in SCD."

The researchers then studied septic shock—another serious blood disorder in which activated, pro-inflammatory neutrophils play a role. Sepsis affects more than one million Americans each year and kills up to half of them. To induce septic shock, control and microbiota-depleted mice received a dose of a bacterial toxin that would normally be lethal. The control mice exhibited the neutrophil aggregates and clumping of neutrophil DNA that contributes to death from septic shock; but the microbiota-depleted mice were largely free of neutrophil complications and survived.

"Remarkably," said Dr. Frenette, "we could prevent microbiota-depleted mice from surviving septic shock if we infused them with aged neutrophils but not if we infused the same number of young neutrophils. So depleting the microbiota may help against inflammatory blood diseases in addition to SCD."

Finally, the researchers investigated whether their findings in mice might be relevant to people with SCD. With help from the Sickle Cell Disease Program at the Children's Hospital at Montefiore (CHAM), they obtained blood samples from nine healthy children and from 34 patients with SCD: 11 patients were taking penicillin daily to ward off infections,

as is recommended for children with SCD age five or younger; the other 23 patients with SCD had been off penicillin for at least two months.

Consistent with the findings in SCD mice, children with SCD not taking penicillin had many more circulating aged neutrophils compared with healthy children who served as controls. The researchers then compared neutrophil levels in two groups of children with SCD—those taking penicillin and those not on the drug—and found a much lower number of aged neutrophils in the blood of those who were taking penicillin.

"Daily penicillin for patients with SCD younger than five works really well in preventing infections," said Dr. Frenette. "Our study suggests that penicillin and other antibiotics could play an even broader role in potentially benefiting older patients. In collaboration with Deepa Manwani, M.D., who directs the CHAM's Sickle Cell Disease Program, we hope to carry out a clinical trial to determine whether antibiotics can help patients with SCD by preventing the sickle cell crisis and long-term organ damage associated with the disease."

More information: Neutrophil ageing is regulated by the microbiome, *Nature*, DOI: 10.1038/nature15367

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