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Anti-tumor antibodies could counter atherosclerosis, study finds

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Investigators at the Stanford University School of Medicine have learned the signal that tumor cells display on their surfaces to protect themselves from being devoured by the immune system also plays a role in enabling atherosclerosis, the process underlying heart attacks and strokes.

A biological drug capable of blocking this so-called "don't eat me" signal is now being tested in clinical trials in cancer patients. The same agent, the investigators found, was able to prevent the buildup of atherosclerotic plaque in several mouse models of cardiovascular disease. If this success is borne out in human studies, the drug could be used to combat cardiovascular disease—the world's No. 1 killer—and do so by targeting not mere risk factors such as high cholesterol or high blood pressure, but the actual lesions bearing direct responsibility for cardiovascular disease: atherosclerotic plaques.

"It seems that heart disease may be driven by our immune system's inability to 'take out the trash,'" said Nicholas Leeper, MD, associate professor of vascular surgery and of cardiovascular medicine.

A study describing the researchers' findings will be published July 20 in *Nature*. Leeper is the senior author.

Atherosclerosis is caused by the deposition of fatty substances along arterial walls. Over the years, these substances form plaques. It's now known that numerous dead and dying cells accumulate in atherosclerotic plaques, which inflammation renders brittle and vulnerable to rupture,

the ultimate cause of heart attack and stroke.

Immune cell malfeasance

Contributing to the pathology is malfeasance on the part of a class of immune cells that first arrive at the site with presumably benign intentions, said Leeper.

"Even a perfectly healthy body turns over more than 100 billion cells a day, every day," he said. "One of the several jobs performed by immune cells called macrophages—from the Greek words for 'big eater'— is to come and gobble up those dead and dying cells, which might otherwise begin releasing substances that can foster inflammation."

Many cells in the human body feature a "don't eat me" signal on their surface: a protein called CD47. The protein tells the immune system that a cell is alive, still going strong and part of a person's healthy tissue.

Normally, as a cell approaches death, its CD47 surface proteins start disappearing, exposing the cell to macrophages' garbage-disposal service. But atherosclerotic plaques are filled with dead and dying cells that should have been cleared by macrophages, yet weren't. In fact, many of the cells piling up in these lesions are dead macrophages and other vascular cells that should have been cleared long ago.

"The fact that there are so many dying cells in an atherosclerotic plaque, although those sick cells are supposed to be cleared promptly by macrophages, got us thinking," said Yoko Kojima, MD, PhD, a basic life science research associate who is the study's lead author.

CD47 in atherosclerotic tissue

In the new study, Leeper, Kojima and their colleagues performed genetic analyses of hundreds of human coronary and carotid artery tissue samples collected at Stanford and at Sweden's Karolinska Institute. They found that CD47 is extremely abundant in atherosclerotic tissue compared with normal vascular tissue, and correlated with risk for adverse clinical outcomes such as stroke.

Much of what's now known about CD47's function stems from pioneering work by Irving Weissman, MD, professor of pathology and of developmental biology and director of Stanford's Institute of Stem Cell Biology and Regenerative Medicine and the Ludwig Cancer Stem Cell Institute. In the late 1990s and early 2000s, Weissman and his colleagues first identified CD47 as being overexpressed on tumor cells, which helps them evade destruction by macrophages. Weissman's group went on to show that blocking CD47 with monoclonal antibodies that bind to and obstruct the protein on tumor cells restores macrophages' ability to devour those cells. Phase-1 clinical safety trials of CD47-blocking antibodies in patients with solid tumors and blood cancers are now underway.

Alerted to the Leeper lab's discovery, Weissman, a co-author of the new study, provided anti-CD47 antibodies so Leeper's group could test their efficacy in battling atherosclerosis.

In a laboratory dish, anti-CD47 antibodies induced the clearance of diseased, dying and dead smooth muscle cells and macrophages incubated in conditions designed to simulate the atherosclerotic environment. And in several different mouse models of atherosclerosis, blocking CD47 with anti-CD47 antibodies dramatically countered the buildup of arterial plaque and made it less vulnerable to rupture. Many mice even experienced regression of their plaques—a phenomenon rarely observed in mouse models of cardiovascular disease.

Looking at data from other genetic research, the scientists learned that surplus CD47 in atherosclerotic plaques strongly correlates with elevated levels, in these plaques, of a well-known inflammation-promoting substance called TNF-alpha. Further experiments showed that TNF-alpha activity prevents what would otherwise be a progressive decrease of CD47 on dying cells. Hence, those cells are less susceptible to being eaten by macrophages, especially in an atherosclerosis-promoting environment.

A vicious circle?

"The problem could be an endless loop," said Leeper, "in which TNF-alpha-driven CD47 overexpression prevents macrophages from clearing dying cells in the lesion. Those cells release substances that promote the production of even more TNF-alpha in nearby cells."

Leeper and Weissman said they hope to find out, in clinical trials of human patients, whether CD47-blocking antibodies will prove effective in breaking that vicious circle.

"This opens up the door for these antibodies' use in noncancerous pathological states where cell proliferation is a primary attribute of the diseased cells," said Weissman, who is the Virginia and D.K. Ludwig Professor for Clinical Investigation in Cancer Research.

One side effect of anti-CD47 antibodies in the mouse experiments, Leeper said, was transitory anemia. "Young red blood cells have high surface levels of CD47, which tells macrophages to leave them alone. Older red blood cells lose this protection, allowing macrophages to cull them from the herd," he said. Anti-CD47 antibodies render these older cells more prone to macrophage attack. But the anemia appeared to clear up fairly quickly in the mice as their bodies adapted by producing numerous fresh red blood cells with high surface CD47 levels.

The work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

Leeper and Weissman have filed a patent describing inhibition of CD47 as a method to prevent atherosclerosis. Both researchers hold equity in Palo Alto-based Forty Seven Inc., a company they cofounded that has licensed related intellectual property from Stanford's Office of Licensing Technology for cancer applications.

More information: CD47-blocking antibodies restore phagocytosis and prevent atherosclerosis, *Nature*, DOI: 10.1038/nature18935

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