Glucose-guzzling immune cells may drive coronary artery disease, Stanford study finds

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Hyper-aggressive immune cells parked in arterial plaque and bingeing on glucose appear to be major drivers of coronary artery disease, Stanford University School of Medicine investigators have found.

The discovery, detailed in a study to be published online Feb. 29 in *The Journal of Experimental Medicine*, could lead to new therapeutic interventions that provide some protection from the disease, which is the No. 1 cause of death in America.

"We've pinpointed a defect in glucose metabolism by a class of arterial-plaque-associated immune cells as a key factor driving those cells into a hyper-inflammatory state," said Cornelia Weyand, MD, professor and chief of immunology and rheumatology, who is the study's senior author. The lead author is postdoctoral scholar Tsuyoshi Shirai, MD, PhD.

Blocking that glucose overconsumption or, for that matter, a couple of other downstream links in the chain of ensuing biochemical events, prevented this hyper-inflammatory activation, the researchers discovered.

The findings support a growing recognition that it's not just arterial deposition of fatty materials called lipids that causes coronary heart disease, but also underlying chronic inflammation. "It's been unclear where the inflammation comes from," Weyand said.

The puzzle of heart attacks

Coronary artery disease, which accounts for nearly half of all deaths in the United States, arises when blood flow through the arteries that supply oxygen-rich blood to the heart is impaired. The underlying process—the buildup of plaque inside the arteries—is called atherosclerosis.

"Most of us develop arterial plaque over the course of our lifetimes," Weyand said. Plaque accumulation can begin early in life, with deposits sometimes evident in individuals as young as 15 to 20 years old, and progresses steadily with advancing age.

When these deposits become severe enough, they can restrict blood flow. It used to be thought that this occlusion triggered heart attacks. But a puzzle remained: If this process is so gradual, why are heart attacks so sudden?

While lipids are a prime constituent of arterial plaque, it's now understood that plaque also contains immune cells—chiefly, a type called macrophages. These cells wear many hats. They attack and ingest invading bacteria, repair tissue, clean up detritus left behind after injury or infection, and more.

"We can't live without them," said Weyand.

Macrophages generally fall into two broad categories: The kinder, gentler ones—so-called M2 macrophages—are like construction engineers, nibbling cellular debris, releasing factors that encourage new cell growth and stimulate blood flow, and otherwise overseeing tissue repair.

So-called M1 macrophages, on the other hand, are inflammatory: As hard-boiled as traffic cops who've heard every excuse, they blow the

whistle on pathogens, recruiting other types of immune cells to the scene. In addition, they attack the invaders themselves by spitting out nasty little clouds of biohazardous chemicals called free radicals. And they squirt out proteins that act both locally and systemically to ramp up the entire immune system to high-alert status.

Testing for inflammation

"Some believe that coronary artery disease patients' macrophages are so preoccupied with their inflammatory power trip they neglect their clean-up tasks," allowing plaque to continue building up in arteries, Weyand said. In any case, the unremitting inflammation renders the plaques increasingly brittle, sometimes culminating in a piece of plaque suddenly breaking off and wounding the artery wall. The ensuing speedy formation of a clot can trigger a heart attack.

The presence of even mildly elevated levels of systemic inflammation can be revealed in laboratory blood tests, such as the one for C-reactive protein, or CRP, now routine in checking cardiovascular health. Statin drugs, which substantially slow the progression of coronary artery disease and prevent heart attacks, are now understood to work not just by lowering lipid levels but also by reducing systemic inflammation. That's why statins reduce heart-related problems among patients who have normal blood-lipid levels but high blood levels of CRP.

The CRP test, in turn, is a good proxy for another protein with a starring role in driving inflammation throughout the body: an immune-signaling protein known as interleukin-6. The body's premier producers of IL-6 are M1 macrophages.

Born in the bone

Macrophages begin life as cells called monocytes that are born in the body's bone marrow. Released into the circulation, monocytes travel through the bloodstream until, responding to chemical "danger signals" that indicate possible injury or infection, they slip into a tissue, take up residence and differentiate into macrophages.

Weyand and her associates commenced their study by comparing monocytes harvested from the blood of 140 patients with coronary artery disease, each of whom had experienced at least one heart attack, with those from 105 healthy, demographically matched control subjects. The blood was obtained by study co-authors John Giacomini, MD, chief of cardiology at the Veterans Affairs Palo Alto Health Care System and professor of medicine at Stanford, and assistant professor of medicine Themistocles Assimes, MD, PhD.

The scientists cultured the monocytes and used standard laboratory methods to differentiate them into macrophages. They observed that the monocytes from patients with coronary artery disease had a pronounced predisposition to develop into inflammatory, IL-6-producing M1 macrophages.

"We also found that macrophages from people with Type 2 diabetes, hyperlipidemia or hypertension—each of these a known risk factor for coronary artery disease—were making more IL-6," said Weyand. The greater the number of these risk factors they had, the more IL-6 their macrophages made, she said.

"Even before taking up residence in arterial plaque and becoming full-fledged macrophages, these patients' monocytes were already leaning toward becoming inflammatory," Weyand said. "If you simply overfeed normal monocytes or macrophages, they don't turn in into high IL-6 producers. We wondered why."

In a series of follow-up experiments, she and her colleagues discovered the reason.

Lots of free radicals

First, they noticed that levels of free radicals inside patient-derived macrophages were double those of macrophages derived from healthy subjects. They traced these free radicals' production to compartments within the patients' macrophages known as mitochondria, which abound in all living cells and serve as their powerhouses, supplying energy by burning sugars and fatty acids.

"This is no easy task," Weyand said. "In doing so, the mitochondria inevitably wind up generating free radicals. The harder they work, the more free radicals they produce."

When Weyand and her associates used a drug to mop up free radicals in the mitochondria of patient-derived macrophages, their IL-6 production—a major feature of their inflammatory prowess—dropped off considerably.

Blocking glucose metabolism within the mitochondria had the same effect. "Something in there is leading to excessive IL-6 production," said Weyand. "That something is our old friend sugar."

The researchers pinned the excessive free-radical production in the mitochondria of patient-derived macrophages to excessive uptake of glucose by those cells, attributable to a faulty overproduction of proteins responsible for importing glucose into cells.

"The primary problem, we learned, is that these macrophages take up glucose at a higher rate than normal cells do," said Weyand. "That causes them to break it down faster, overheating their mitochondria, which then

produce too many free radicals."

Surprising finding

One of the most surprising findings was the discovery that those high levels of free radicals were inducing a change in the status of PKM2, an enzyme that normally busies itself by helping to generate energy from the breakdown of glucose. Under the free-radicals' influence, the scientists learned, PKM2 blows off its day job and instead heads into the cell nucleus, where it activates a protein called STAT3 that proceeds to substantially boost the production of pro-inflammatory cytokines, including IL-6.

In the lab, the scientists tested a drug designed to prevent PKM2's status change and succeeded in lowering the IL-6 output of patient-derived M1 macrophages.

The good news, Weyand said, is that several interventions—blocking glucose uptake, sponging up free radicals and preventing PKM2's status change—reduce the macrophages' excess inflammatory activity. This could lead to new therapeutic approaches, she said.

The team's 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.

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