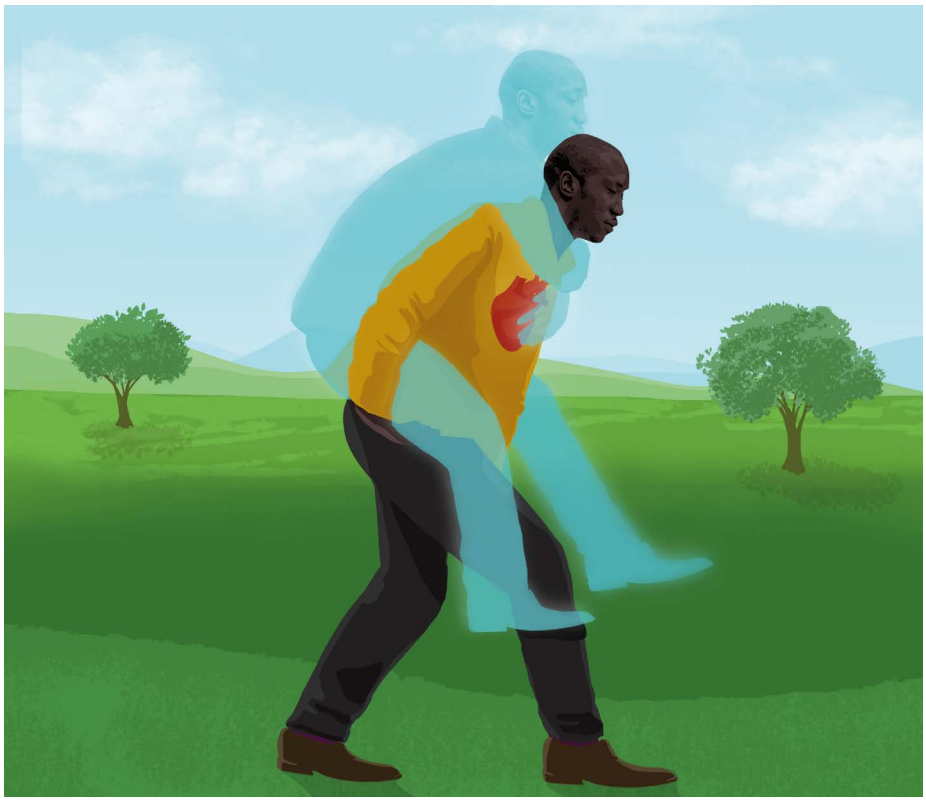

SAM FALCONER

The ghost of immunity past

A primed state adopted by innate immune cells has revealed disease mechanisms and drug targets for atherosclerosis. **By Amanda B. Keener**

Most cardiovascular events begin with atherosclerosis, a condition in which inflammation and fatty deposits cause arteries to narrow and stiffen. These deposits, or plaques, build up over years. When they dislodge and travel to smaller vessels, they can block blood flow to vital organs, causing heart attacks and strokes.

Although many of the contributing factors for atherosclerosis, including high cholesterol and hypertension, are well understood, addressing them doesn't eliminate the risk of developing the disease. "Lipid-lowering medications only reduce the cardiovascular-disease risk by about 35%, so there is this huge gap of things we leave untreated," says Esther Lutgens, who studies vascular immunology at the University of Amsterdam. In the past few decades, she says, researchers have learnt that addressing inflammation might be a way to fill that gap. After all, cardiovascular disease is more common in people with chronic inflammatory diseases such as rheumatoid arthritis and chronic infection of the gums.

Now, a type of immune memory — called trained immunity — is offering researchers a biological mechanism to explain some of these links, as well as suggesting new drug targets.

Trained immunity occurs in innate immune cells. These cells don't form a memory in the classic sense — by making receptor proteins that bind to specific pathogens. Instead, they remember previous exposures through changes to their genes and metabolism. "When these cells encounter a stimulus in the future, whether related or unrelated to the original one, they can respond faster and stronger," says George Hajishengallis, an immunologist at the University of Pennsylvania in Philadelphia.

Researchers have shown that pathogens, stress hormones and cholesterol can all train immune cells that are known to play major parts in atherosclerosis. Now they are testing whether trained immunity could be a mechanistic link between inflammatory diseases and atherosclerosis. "This really gives a mechanism that can explain some of the previous findings of how risk factors lead to a long-term effect in atherosclerosis. And a very exciting framework that can be used in the future to develop novel drugs," says Niels Riksen, a vascular medicine specialist at Radboud University in Nijmegen, the Netherlands.

Trained to inflame

Riksen works closely with the team of researchers at Radboud who initially described trained immunity ten years ago[1]. They found that exposure to certain fungi or bacteria caused innate immune cells, including monocytes and macrophages, to acquire epigenetic marks on their histones — changes to the proteins that spool and store DNA, but that do not involve alterations to the underlying DNA code. These modifications allowed the cells to rapidly access key genes involved in inflammation and metabolism and mount fast, heightened immune responses to any stimulus later on.

A decade of work has since shown how trained immunity protects cells, animals and people from infection. But from the very beginning, Riksen suspected that this phenomenon might not be unequivocally beneficial. After all, monocytes and macrophages exacerbate atherosclerosis. When monocytes encounter an atherosclerotic plaque on the side of a blood vessel, they morph into macrophages and release inflammation-inciting molecules called cytokines. Macrophages also release enzymes that eat away at protein and cause plaques to crumble into pieces that can block blood flow. In this context, Riksen thought, hyper-responsive trained monocytes or macrophages could be harmful.

In 2012, Riksen enlisted then-graduate student Siroon Bekkering, an immunologist at Radboud, to look for connections between trained immunity and atherosclerosis. Bekkering started by dosing human monocytes with two types of cholesterol: low-density lipoprotein (LDL), often called 'bad' cholesterol, and its oxidized form, oxLDL. She found that oxLDL induced epigenetic changes that enabled the cells to make more cytokines after they had been exposed to an unrelated immune-triggering compound. That is, cholesterol could induce trained immunity — at least in cells[2].

To test this in people, the team analysed monocytes from 20 individuals with severe atherosclerosis[3]. The cells had all the hallmarks of trained immunity, including heightened cytokine responses, epigenetic changes and alterations to metabolism-related genes. A

study published by another group in the same year reported similar results[4].

The pieces were falling into place. Riksen says some of the most direct evidence came from studying people with familial hypercholesterolemia, a genetic condition causing high levels of blood cholesterol. Bekkering found that monocytes from 25 people with this condition had telltale characteristics of training that persisted for three months after they starting taking cholesterol-lowering drugs called statins[5]. The condition increases cardiovascular-disease risk, and that risk does not completely disappear with statins. Riksen says it's possible that trained immunity maintains the inflammation long after treatment begins. The team is following the study participants with the hope of finding out whether trained immunity can be reversed or prevented.

Inflammatory legacy

Monocytes circulate for only a few days before they're cleared from the body and replaced. So for trained immunity to last for months, it must also affect monocyte precursor stem cells in the bone marrow. Over the past few years, research in mice and people has shown that the tuberculosis vaccine induces trained immunity in bone marrow stem cells that lasts for at least a year.

Last year, Riksen's team found evidence that the same might be true for coronary artery disease. The researchers analysed bone-marrow samples from 13 people with severe atherosclerosis and found that some inflammatory genes were more active in the monocyte precursor stem cells of these 13 people than in those of healthy controls[6]. They also found changes to the stem cells' metabolism that were consistent with trained immunity. The team is now examining the cells' histone modifications.

The concept that trained immunity could affect stem cells offers a compelling backdrop for the links between atherosclerosis and inflammatory conditions, such as chronic gum disease and rheumatoid arthritis. Cytokines produced as a result of these conditions can easily reach the bone marrow, Hajishengallis says, theoretically resulting in a long-lived supply of trained monocytes that can contribute to inflammation anywhere in the body.

"It's a concept with a great explanatory power," Hajishengallis says. Although scientists had already made many of the key observations that point to trained immunity, "we didn't know how to connect the dots," he says. Hajishengallis's team is now testing whether periodontitis, a severe infection of the gums and jawbone, can induce trained immunity and contribute to atherosclerosis in mice.

Trained immunity might also explain why past inflammation increases heart-disease risk. David Burgner, a paediatric infectious-disease specialist at Murdoch Children's Research Institute in Melbourne, Australia, has found, in large prospective studies, that severe infections early in life increase the risk of atherosclerosis later on. Meeting Bekkering at a conference in 2016, he says, was like finding the missing pieces to a puzzle.

> **"I suspect that a lot of these traditional risk factors are affected through inflammation."**

Burgner and Bekkering joined forces to look for signs of trained immunity following inflammation in the womb and in early life. Up to 70% of premature births involve an infection of the membranes surrounding the fetus, called chorioamnionitis. The researchers mimicked this in mice with a genetic predisposition to atherosclerosis and found that those that had experienced inflammation both before and after birth had much larger atherosclerotic plaques as adults[7]. They are now looking for markers of trained immunity in innate immune cells from umbilical-cord blood donated by mothers with chorioamnionitis who gave birth prematurely.

Burgner is also running several studies to examine how infection fits into the larger picture of atherosclerosis and heart-disease risk. "I suspect that a lot of these traditional risk factors are affected through inflammation, and the amount of inflammation they generate will be a legacy of what's gone before," he says. "I suspect a lot of that early in life is infection."

Cellular untraining

As researchers define the biology behind trained immunity, they are also uncovering potential drug targets. In vaccination and infection studies, trained immunity can be prevented by blocking specific histone modifications or enzymes involved in glucose metabolism. Riksen's team is testing some of these approaches in mouse models of atherosclerosis. Because all cells use glycolysis and modify histones, however, researchers are also looking for ways to target these processes specifically in monocytes and their precursors.

Willem Mulder, a biomedical engineer who moved his laboratory to Radboud in January, is working with nanosized particles made mainly of lipoproteins that are easily engulfed by monocytes and macrophages, and could be used to carry drugs. In 2019, Mulder co-founded biotech firm Trained Therapeutix Discovery in Luxembourg to develop these nanobiologics for use against trained immunity.

Some of the compounds that show promise for blocking trained immunity include inhibitors that reduce interleukin-1β, a cytokine known to have a role in cardiovascular disease, as well as drugs already in use for cardiovascular disease and other conditions – statins and metformin, for example.

So far, the company has focused on using the particles to deliver drugs that either enhance or block trained immunity in cancer or after organ transplants, but he says that the same technology can be adapted for cardiovascular disease. He predicts that its greatest impact will come when used after a heart attack, stroke or severe infection to prevent trained immunity and thereby lower the risk of future cardiac events. In collaboration with the US National Institute of Allergy and Infectious Diseases, Mulder's team is now testing whether drugs that prevent trained immunity can reduce the risk of cardiac events following infection with the virus SARS-CoV2 in rhesus macaque monkeys with high cholesterol.

The drugs that prevent immune training won't necessarily reverse it, Bekkering says. Indeed, her study involving people with hypercholesterolemia suggests that statins can't undo training in human cells. There are hints that other interventions could undo trained immunity. In one study[8], Riksen and his colleagues ran a 16-week programme that encouraged people who were 55 or older to increase their physical activity. When they tested the participants' monocytes in the lab at the end of the programme, the team found that the cells produced lower levels of cytokines than before. However, the study was small – just 16 people – and the team is still testing whether the intervention altered epigenetic changes or other signs of trained immunity.

Whether the goal is prevention or reversal, trained immunity is allowing new approaches. "I think there's an entire field of drug study open to really tackle this trained immunity perspective in atherosclerosis," Lutgens says. The hopeful prognosis is that this branch of the immune system could be stripped of its potentially harmful effects without losing all the benefits that it brings along.

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