INFLAMMATION OVERLOAD

New evidence suggests friendly fire from the immune system is at the heart of common diseases. DYANI LEWIS takes a closer look. →

IT WAS A PATIENT who thought he was having a heart attack that inspired Mark Nidorf. The man had come into the Perth cardiologist's surgery complaining of pains that felt like his heart was being squeezed in a vice. Nidorf diagnosed pericarditis, acute inflammation of the membrane encasing the heart. He prescribed an old drug called colchicine, one ancient Egyptians used to alleviate gout.

"I GAVE HIM a single dose, and the next day he felt fantastic," Nidorf recalls a decade later. The incident got him thinking about other patients, who had experienced real heart attacks because of blocked arteries.

Might inflammation also be involved here, and if so could colchicine be helpful?

Nidorf signed up over 500 of his heart-disease patients for a clinical trial. All continued with their usual panoply of heart medications – cholesterol-lowering statins and clot-busting aspirin among them. Half added a low dose of colchicine to the mix. "My colleagues thought I was crazy."

After three years, Nidorf's gamble paid off. In patients taking colchicine, only 4.5% experienced a stroke or heart attack. For the others, the rate was nearly four times as high – 16%.

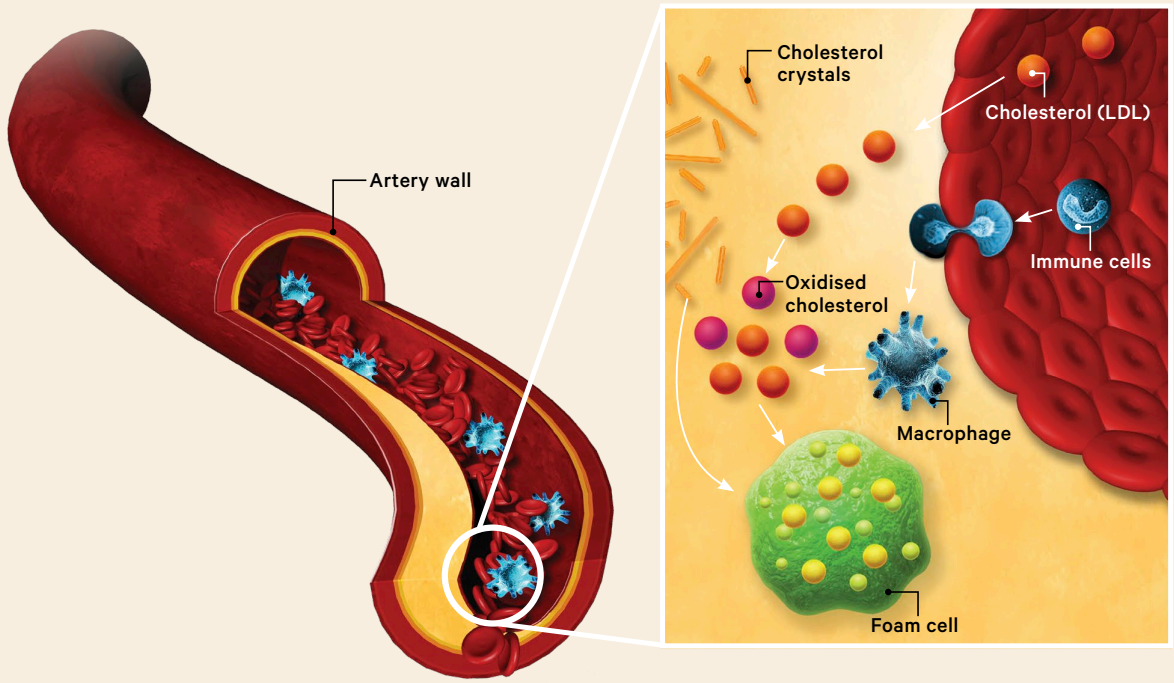
It was a stunning result, with potentially huge ramifications for the treatment of heart disease, the leading cause of death worldwide. And with it, Nidorf became a committed convert to the inflammation theory of disease, joining a small but growing band of medical practitioners who believe that the excessive response of the body's immune forces to perceived threats is intimately involved in common diseases. Proving this theory would transform the way medicine treats them.

But proof of the inflammation theory requires evidence far more compelling than Nidorf's small trial.

The game changer may well be a vast clinical trial that goes by the acronym of CANTOS: the Canakinumab Anti-inflammatory Thrombosis Outcomes Study. Published in the prestigious *New England Journal of Medicine* (NEJM) in September 2017, 10,000 patients who had suffered a heart attack were given different dosages of an anti-inflammatory drug or a placebo. After 3.5 years, those receiving the drug had fewer heart attacks and strokes.

An unexpected bonus was that they were 67% less likely to develop lung cancer.

The CANTOS findings offer compelling vindication for devotees of the inflammation theory. The cancer outcomes in particular add weight to the suspicion that inflammation is at the heart of many debilitating conditions including diabetes, Alzheimer's disease and even depression.



02 | Plaques arise when macrophages from the bloodstream enter the artery wall, gobble up cholesterol (LDL) and turn into fatty foam cells. Oxidised cholesterol and cholesterol crystals elicit the greatest inflammatory response.

Derek Richard, who studies cancer and ageing at Queensland University of Technology, sums up the new perspective: "Inflammation is the source of all evils."

Nidorf has found himself recast as a pioneer. "It's gone from 'What the hell are you doing?' to 'Wow, this is really interesting and we're going to fund it'," he says, noting Australia's National Health and Medical Research Council has funded the latest colchicine trial of 5,000 patients.

But old drugs like colchicine are just a placeholder. A pharmaceutical gold rush is on to develop high-tech drugs that finely recalibrate the operations of our immune defence forces.

THE IMMUNE SYSTEM is here to protect us. But like any army, it can go awry. Instead of the 'shock and awe' campaign that typifies a healthy immune response to infection or injury, we end up with a protracted 'Afghanistan'.

What's emerged in recent years is that the type of campaign waged depends on the intelligence relayed at the battle scene.

The immune army's intelligence comes in the form of chemical emissaries called cytokines. Released by particular regiments of the immune army, pro-inflammatory cytokines call out the troops. Anti-inflammatory cytokines send them home. It is this balance of intelligence signals that decides between a healthy campaign that lasts days, or the debilitating chronic inflammation that can last for years.

MEDICAL SCIENTISTS HAVE long been aware of a link between heart disease and inflammation. In the 1850s German pathologist Rudolf Virchow reported that the fatty plaques seen in heart disease bore the classic signs of inflammation.

We now know the workings of the botched immune response that turns cholesterol into these plaques. When blood-borne cholesterol, packaged as low density lipoprotein (LDL), is deposited in the walls of arteries, immune foot soldiers called macrophages stream in to gobble it up. If the cholesterol oxidises or forms crystals, the macrophages rile up and release signals called cytokines that recruit more immune cells. Some fortify the artery with calcium deposits; others, fibrocytes, lay down connective fibres. It is a diabolically complex repair job that either produces a stable scab or, more dangerously, an unstable pus-filled pimple, ready to burst and form an artery-plugging clot.

Despite the known association between inflammation and cardiovascular disease, decades of using common anti-inflammatory drugs showed no evidence of benefit; they actually made things worse. The most notorious example is Vioxx, an anti-inflammatory drug that was widely used to treat

arthritis and pain. It was withdrawn in 2004 after it was shown to increase the risk of heart attacks and strokes. On the other hand there was incontrovertible evidence that cholesterol-lowering drugs like statins reduced hearts attacks and strokes. As a result, says cardiologist Thomas Marwick, director of the Baker Heart and Diabetes Institute in Melbourne, the inflammatory theory "sat in no-man's land".

PAUL RIDKER WAS determined to get some movement with the theory. Now director of the Center for Cardiovascular Disease Prevention at Boston's Brigham and Women's Hospital, he began his career planning to specialise in infectious diseases.

He's been interested in the inflammation theory almost as long as he has been a cardiologist, even though it was distinctly unpopular. "For the better part of 30 years, I've often felt at professional meetings that I'm walking around with a target on my back," he confides.

Besides the disheartening findings with anti-inflammatory drugs like Vioxx, there was the chicken and egg problem. Was inflammation a cause of heart disease, or merely a consequence of it?

In the mid-1990s Ridker set out to find the answer in a cache of frozen blood samples stored at Harvard Medical School. A decade earlier, they had indicated aspirin could almost halve the risk of heart attack. Ridker suspected they had more secrets to reveal.

The blood samples came from 22,000 healthy male physicians aged 40–82 who had participated in the Physicians' Health Study. The goal of the study, which began in 1982, was to see if aspirin or the antioxidant beta-carotene could prevent cardiovascular disease or cancer. Participants were randomly assigned to receive 325 mg of aspirin, 50 mg of beta-carotene, both, or neither. The clear benefits of taking aspirin were visible within five years.

But did aspirin lower heart attack risk simply by stopping blood clots, or was it also working to prevent inflammation? Ridker decided to find out by thawing more than 1,000 samples and testing them for a marker of inflammation called high sensitivity C-reactive protein (hs-CRP). Released by the liver, its role is to seek bacteria or damaged cells and mark them for destruction . Because levels of hs-CRP in the blood can rise 100-fold during an infection or injury, it is also a sensitive measure of inflammation.

Ridker found that hs-CRP levels were also potent augurs of cardiovascular disease. Compared to physicians with the lowest levels of hs-CRP, those with the highest levels were three times as likely to have a heart attack and twice as likely to have a stroke.

"It demonstrated that inflammation precedes, by many years, first-ever heart attacks, strokes and cardiovascular deaths," says Ridker. Moreover, physicians with the highest levels of hs-CRP benefited most from taking aspirin. The findings, published by Ridker in 1997 in the NEJM, strongly suggested that aspirin was working by lowering inflammation.

Another clue came from interrogating the actions of statins. It was known that besides lowering cholesterol, statins also reduced hs-CRP levels. Was this contributing to their protective effects against heart disease? Two papers published in NEJM in 2005 and 2008 suggested it was. Irrespective of changes to cholesterol, patients whose hs-CRP levels dropped after statin therapy had fewer heart attacks and strokes.

The findings all suggested that lowering inflammation offered protection. But to prove it, Ridker needed an intervention that exclusively targeted inflammation. He seized on canakinumab, a drug already on the market for treating juvenile arthritis. Operating like a 'narrow scalpel', the drug is an antibody that neutralises a single inflammatory molecule – called IL-1β – without touching a person's cholesterol levels or blood-clotting ability.

To test canakinumab, the CANTOS trial enrolled people who'd already had a previous heart attack and had high levels of hs-CRP. Their cholesterol levels were normal, because they were all being aggressively treated with statins. For three and a half years, participants received infusions of canakinumab or a placebo every three months. By the trial's end, those on the highest dose were 15% less likely to suffer a heart attack or stroke, and 30% less likely to require the unblocking of an artery through stenting or cardiac bypass surgery.

"As a proof of principle, it's huge," says Ajay Chawla, of the University of California San Francisco, who studies the role of inflammation in type 2 diabetes.

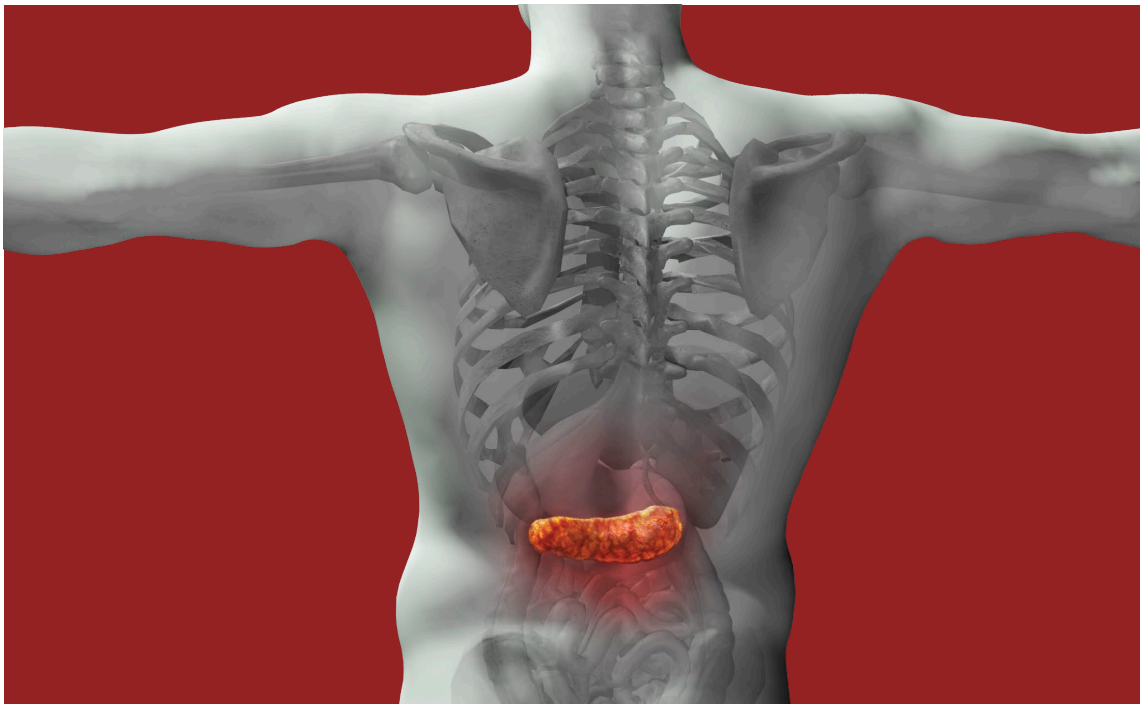
By proving at last what Virchow suspected over 160 years ago, the CANTOS trial has been a game changer.

Not surprisingly, scientists working on other common maladies are keen to follow in its footsteps. In each case, the challenge is to clearly incriminate inflammation as the villain behind the disease.

LIKE HEART DISEASE, type 2 diabetes (T2D) is a common disease of ageing. But the question of whether chronic inflammation drives the disease remains highly controversial.

In healthy people, blood sugar levels are tightly controlled. After a meal, sugars are rapidly ferried out of the blood into body tissues, thanks to the driving action of the pancreatic hormone insulin. That's not the case in diabetics. Their blood sugar levels remain high.

The reason is two-fold. First the body tissues



The jury is out on whether reducing inflammation will make a difference to type 2 diabetes, a disease that damages the pancreas.

resist the influx of sugar, developing so-called 'insulin resistance'. Second, to overcome that resistance, the pancreas goes into overdrive pumping out more and more insulin. Eventually the pancreas becomes exhausted and its insulin-producing beta cells die. Diabetics often end up dependent on insulin injections.

There is a lot of circumstantial evidence that inflammation can hack the control of blood glucose. During an infection, for instance, blood glucose levels stay high. Another key insight has come from a closer inspection of fat. Obesity is a major risk factor for T2D, but exactly why hasn't been clear.

In 1993, Gökhan Hotamisligil, an endocrinologist at Harvard, offered an answer that placed inflammation front and centre. He found that fat cells, when engorged with fat, behaved as if they were infected, releasing cytokines like TNF-α that call up an immune response. Not only did this cause the fat tissue to be infiltrated by immune cells, TNF-α also generated insulin resistance in the surrounding tissues.

The finding gave birth to the neologism 'metaflammation', the idea that fatty tissue provokes inflammation which in turn leads to the metabolic diseases of insulin resistance and T2D. It also helped explain why so many other diseases rise in lockstep with obesity. Inflamed fatty tissue releases cytokines into the bloodstream, recruiting legions of riled-up immune cells that can wreak collateral damage on various tissues of the body.

Diabetes researchers were particularly intrigued by the finding because it suggested that disrupting the cytokine broadcast could halt the progression to diabetes. Among the researchers who've spent years trying to do that is Mark Febbraio at Sydney's Garvan Institute. Febbraio's studies of obese mice found that insulin resistance precedes the development of inflammation, not the other way around. And treating mice with anti-inflammatories did not stop their progression to T2D.

The CANTOS trial also showed no signs that reducing chronic inflammation reduced the risk of developing diabetes. "The evidence that blocking inflammation can prevent insulin resistance and diabetes simply isn't there," says Febbraio.

Chawla doesn't agree and suggests canakinumab may have been too fine a scalpel. Perhaps a blunter instrument will work better? Last year a small clinical trial based at Joslin Diabetes Center in Boston reported that an aspirin-like drug called salsalate effectively lowered blood sugar levels .

"We cannot possibly put all the nails in the coffin [of the inflammatory hypothesis] by saying IL-1β did not do the trick," says the Joslin Institute's Steven Shoelson, a co-author of the trial.

A CLOSER LOOK

THE ANTI-INFLAMMATORY INDUSTRY

A QUICK BROWSE of the internet reveals a slew of advice that promises to keep inflammation – and disease – at bay. But as antioxidant superfoods and colonics make way for anti-inflammatory diets and hyperbaric oxygen therapy, it can be difficult to distinguish fad from fact.

There's no doubt that diet can influence inflammation. That's because what we eat determines the make-up of the population of bacteria and other microbes that inhabit our gut – the microbiome. When this ecosystem is thrown off kilter – through poor diet or antibiotic use – the protective mucous-covered lining of the gut becomes 'leaky'. Bacterial fragments can then seep into our bloodstream where they rouse the immune army. Chronic inflammation ensues.

So-called 'anti-inflammatory' diets steer people away from sugary and fatty foods, towards a diet rich in fruit and vegetables, wholegrain cereals, olive oil, nuts and fish. Often referred to as the 'Mediterranean diet', studies have indeed demonstrated that it reduces inflammatory markers such as hs-CRP.

The fibre in 'anti-inflammatory' diets is particularly beneficial at lowering levels of hs-CRP. That's because fibre promotes a tightly sealed gut lining by encouraging the growth of particular microbes that produce short chain fatty acids. These molecules don't just influence gut health. They are intrepid travellers and in the brain, they glue together the cells of the blood-brain barrier , much as they do the intestinal lining. This protects the brain from bacterial detritus floating around the body, and may be why fibre is associated with lower rates of depression – another condition linked to inflammation.

There's evidence that some foods increase inflammation. These include charred meats, saturated fats and sugar. Indeed, the Mediterranean diet probably quells inflammation as much by what it leaves out as by what it puts in.

Still, there is a paucity of trial data showing that by changing our diet in a particular way, disease is prevented. "It's largely unproven," says Febbraio.

The proven dietary intervention to reduce inflammation is weight loss. Fat tissue behaves like infected tissue, recruiting the immune forces and raising the levels of circulating inflammatory markers.

A more high-tech offering from the anti-inflammatory industry is hyperbaric oxygen therapy (HBO). The idea with HBO is to deliver more oxygen than usual to your body's tissues. This is achieved by breathing 100% oxygen – instead of the usual 21% that's in air – while in a pressurised chamber.

The technique is medically approved for certain situations where tissues are starved of oxygen: in a diver with the bends, in people suffering carbon monoxide poisoning, after radiation therapy and to aid wound healing. It's also used by athletes and even racing camels to aid recovery! By getting oxygen to damaged, oxygen starved tissue, it prevents cell death.

HEREIN LIES the connection with inflammation. Since cell death triggers inflammation, HBO can reduce inflammation in a tissue injury setting.

But does this translate to benefits in the setting of chronic low level inflammation?

"There's no evidence for that," says Andrew Fock, head of Hyperbaric Medicine at Melbourne's Alfred Hospital.

Anti-inflammation clinics promote the use of regular HBO to reduce inflammation as measured by levels of hs-CRP. Fock, however, raises concerns that regular HBO is not necessarily harmless. "In every setting the timing and dose are critical. You've got to consider the pros and cons of every medical intervention."

For instance, researchers disagree about whether HBO does more harm than good for stroke patients because it could raise the levels of harmful reactive oxygen species.

For Nidorf, the idea of undergoing HBO to reduce general inflammation is laughable. He points out that there is a large normal variation in the blood levels of hs-CRF and advises those who are concerned by high levels to get their arteries checked.

"You don't treat a marker, you treat a condition."

Inflammation is linked to many cancers. A new study proves that blocking it can prevent lung cancer.

AS FAR AS THE LINK between cancer and inflammation, the CANTOS findings offer the most compelling evidence to date. People on the highest dose of the anti-inflammatory were 67% less likely to get lung cancer, and 77% less likely to die of the disease than those taking the placebo. It adds to the already considerable body of evidence that inflammation plays a role in cancer.

A chronic infection can place somebody at higher risk of cancer. For instance, human papillomavirus (HPV) infection is linked to genital cancer, hepatitis B and C to liver cancer, HIV to Kaposi sarcoma, and infection with *Helicobacter pylori* raises the risk of stomach cancer. Chronic illnesses like Crohn's disease raise the risk of colon cancer while obesity, an inflammatory state, raises the risk of multiple cancers.

Biologists have no difficulty explaining the mechanism. Chronic inflammation causes cells to produce reactive oxygen species (ROS). These corrosive chemicals kill invaders that have been gobbled up by immune cells. But the ROS also wreak damage on the DNA of host cells that leads to mutations and cancer.

So does taking an anti-inflammatory drug protect against cancer? As far as aspirin and bowel cancer go, we've been watching this space now for over 20 years. A 2005 study found that women who used high dose aspirin (600 mg per day) for over a decade had a reduced risk of bowel cancer. But long term use of aspirin can have major side effects such as brain haemorrhage and abdominal bleeding.

WHEN IT COMES TO Alzheimer's disease (AD), the inflammation hypothesis is riding a wave of popularity. This, in part, is because the last three decades of following the 'amyloid hypothesis' – which holds that crystalline deposits of beta amyloid protein in the brain are the agents of the disease – has proved fruitless.

Again the idea that inflammation might be a key driver is nothing new. In 1901 another German pathologist by the name of Alois Alzheimer sketched numerous microglia, the immune cells of the brain, nestled up to the dying neurons in a slice of diseased brain tissue. Less visible were cytokines like TNF-α that are also detectable in the brain tissue of people with Alzheimer's.

But are the immune forces merely responding to the damage in the brain, or did they help cause it?

Bryce Vissel at the University of Technology Sydney suspects microglia are indeed perpetrating the damage by overdoing part of their normal job: pruning the connections between brain cells or synapses. "My current thinking is that during inflammation, the process goes overboard, resulting in synaptic loss, which is the major hallmark of AD."

The evidence that inflammation drives the damage is building. In 2013, Michael Heneka at the University of Bonn reported that mice lacking a single gene do not go on to develop dementia. It was a telling result because it turns out this gene (NLRP3) plays a crucial role in the intelligence transmissions of the immune army. Microglia set up field transmitters dubbed 'inflammasomes' to call out more troops. Without

Evidence is building that inflammation plays a role in Alzheimer's disease.

NLRP3, they could not assemble the inflammasome.

In 2017, a *Nature* paper from Heneka's group showed how the diverse suspects at the Alzheimer's crime scene may all be part of the same gang – at least in mice. Shredded bits of inflammasome released from microglia seeded crystals of β amyloid. In turn, β amyloid crystals triggered more inflammasomes to pop up inside the microglia. Bottom line: β amyloid crystals and inflammasomes are locked in a vicious cycle that spreads the theatre of battle across the brain. This might explain why boxers and footballers are at higher risk of AD. A minor brain trauma could sew the seed of inflammation that snowballs into the disease.

There is some evidence that blocking inflammation will protect people against AD. In 2001 the Rotterdam study observed nearly 7,000 Dutch people aged over 55 for seven years, and checked the drugs they were taking from highly accurate pharmacy records. It found that using anti-inflammatories like ibuprofen for more than two years reduced the risk of dementia five-fold. But retrospective studies that observe what people did in the past, rather than testing interventions, provide only weak evidence.

Blocking the inflammatory cytokine TNF-α also seems to delay the disease. But so far, "these studies remain controversial", says Vissel, largely because none of the existing drugs are precise enough in their effects.

But that's all rapidly changing. "We're seeing the dawn of highly targeted new treatments," says Vissel and he adds, "it's about time."

WHEN IT COMES to pinpointing the role of inflammation in common diseases, researchers still have a way to go to untangle cause and effect. But there's one cause and effect story that has recently unravelled.

The biggest risk factor for many illnesses is age. It turns out, ageing itself ramps up inflammation in the body. In 2001, Judith Campisi from the Buck Institute in California discovered the source of this rising inflammatory signal: senescent cells. They start as ordinary cells, perhaps forming liver or muscle tissue. But along the way, triggered by damage or disease, some cells go into a state of arrest. They no longer divide but belch out cytokines. Familiar story: cytokines attract the unruly troops that spray friendly fire on surrounding tissue.

In 2011, Jan van Deursen and colleagues from the Mayo Clinic in Rochester, Minnesota, found a way to eliminate senescent cells in ageing mice, increasing their average lifespan by around 25%. Other mouse experiments have since found that pruning away these

senescent cells could restore youthful organs, muscle strength and even regrow hair.

Campisi, van Deursen, and others have co-founded Unity Biotechnology to develop 'senolytics', drugs that seek and destroy senescent cells. The ultimate goal, says Campisi, is a tonic that would be taken every five years to prune away senescent cells.

WITH THE EVIDENCE firming for inflammation as public health enemy number one, the race is on to develop drugs that can tone it down. But don't expect your doctor to be writing you a prescription for a miracle cure any time soon. There is a need for cheaper and safer drugs.

Canakinumab, for instance, clearly reduces the risk of heart attacks and cancer. But it costs US$16,000 per infusion – and you need them regularly. It also has its risks. A small number of people died of infections during the CANTOS trial.

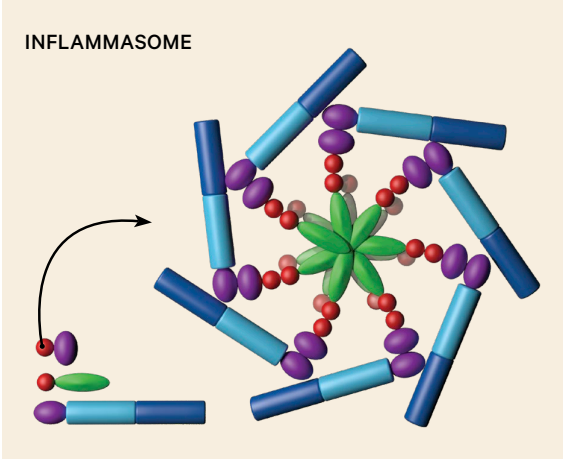
Colchicine now being tested in a large trial by Nidorf, however, has a proven record for safe long term use. People with Familial Mediterranean Fever (FMF), a rare inherited condition where inflammasomes pop up too frequently, take low doses of colchicine for years with few side effects. And as Marwick notes, "while Canakinumab is ridiculously expensive, colchicine is ridiculously cheap".

Ridker, too, is turning to cheaper off-the-shelf alternatives. His latest effort is the Cardiovascular Inflammation Reduction Trial (CIRT). Funded by the US government, he is testing a low dose of the steroid drug methotrexate, already widely used to treat rheumatoid arthritis. Past observation of these patients suggests they had lower rates of heart attacks and strokes. Results are expected in 2021.

DRUG DEVELOPERS ON the other hand are being spurred to develop a new generation of high tech drugs. They have been greatly helped by the discovery of the inflammasome.

While the CANTOS trial proved that quelling inflammation can prevent disease, back in the lab, researchers have been dissecting the fine details of battleground intelligence. The inflammasome offers the chance to recalibrate operations: the goal being to retain the capacity for shock and awe campaigns but eliminate chronic insurgencies.

It turns out there are 14 different inflammasomes that are associated with different immune campaigns. The NLRP3 inflammasome is the one that crops up in chronic inflammatory diseases. It seems to be particularly roused by protein crystals – a common feature of many chronic illnesses. The latest strategy for developers is to target the NLRP3 inflammasome, leaving the 13 other varieties intact to fight infection.



04 | The inflammasome is a transmitter assembled from cellular components by the front line troops of the immune system. It broadcasts cytokines that lead to a chronic inflammatory response. By preventing its assembly, chronic inflammation and diseases may be avoided.

"It's a very, very exciting field," says chemist Matt Cooper from the University of Queensland. "Fifteen years ago, this target didn't even exist."

In 2016, Cooper co-launched the start-up company Inflazome Ltd to target the NLRP3 inflammasome. He estimates there are half a dozen or more start-ups with the same strategy. Some are hitting the big time. Last year pharmaceutical giant Bristol Myers Squibb bought out IFM Therapeutics which is developing inflammasome drugs to treat cancer.

THE FLOOD GATES seem to have opened. Old and new drugs to quell chronic inflammation are rushing to prove their worth in cardiovascular disease, Alzheimer's, cancer and other common diseases.

"The next 20-30 years of cardiovascular medicine will be extraordinary," says Ridker.

In the meantime Ridker offers a simple and cheap prescription for lowering chronic inflammation: watch what wyou eat, and get more exercise.

"All my patients are instructed to put shorts and sneakers in their briefcase," says Ridker.

DYANI LEWIS is a freelance science journalist based in Melbourne, Australia.

ILLUSTRATIONS
Anthony Calvert