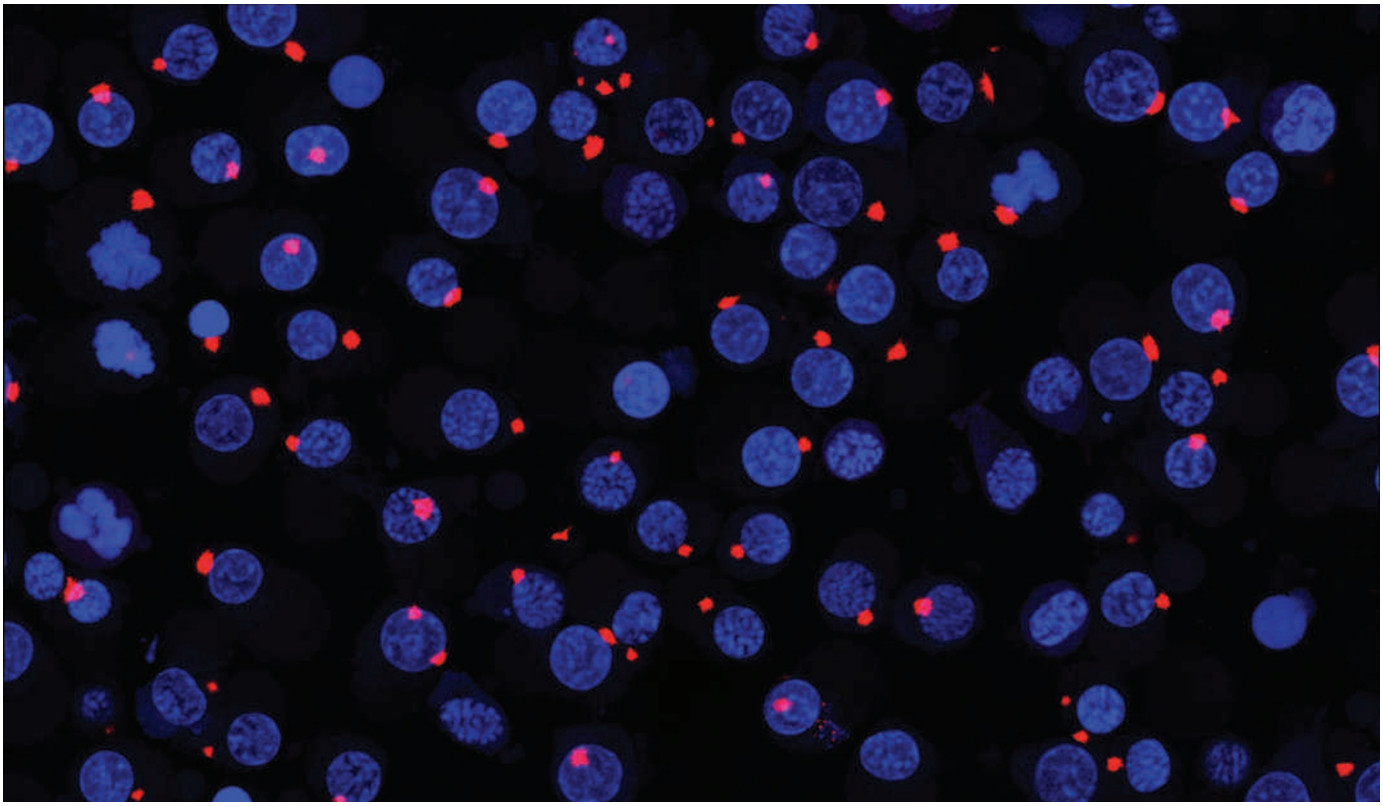

EICKE LATZ

One burning question: why is there only one inflammasome (red dot) per cell (nuclei stained blue)?

INFLAMMATION

A complex problem

Multi-protein inflammasomes are being implicated in a surprising number of diseases, and researchers are keen to find out why.

BY KATHARINE GAMMON

The immune system's primary task — telling friend from foe — is no easy job. Faced with a serious threat such as an infection or wound, the system needs to send defenders out immediately. But it also needs to lie low in the face of innocuous visitors.

When confronted by a dangerous outsider, the human body has two lines of defence. There is learned immunity, which is acquired by exposure to a pathogen, either from the environment or through a vaccine. Then there is innate immunity, which is the immediate hard-wired reaction to outside invaders. The key ingredients in the innate response are inflammasomes — large protein complexes that form in response to a perceived threat, sounding the alarm for the body's inflammatory responses.

A growing body of research indicates that defects in the structure and activity of inflammasomes are central to a vast number of illnesses, from atherosclerosis and arthritis to Crohn's disease, cancer, diabetes and irritable bowel disease. Targeting these complexes could usher in a new age in drug development. But doing so requires precision methods of seeing inflammasomes at work.

SOUNDING THE ALARMIN

Over the past decade, researchers have begun to piece together how inflammasomes work. These large molecular complexes form in response to different stimuli — and their composition can differ accordingly. The stimuli can be incredibly diverse, including bacteria and bacterial toxins but also other 'danger signals' such as cholesterol crystals. Once they have formed, they activate the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18, which then call in lymphocytes to battle the perceived infection.

Scientists once believed that the only job of the innate immune system was to protect the host from external danger. Recently, however, a more nuanced understanding has emerged. It is now clear that the innate immune system — and inflammasomes in particular — can be activated by endogenous molecules known as alarmins, released by damaged cells, or danger-associated molecular patterns. Cholesterol crystals are one example; others are monosodium urate crystals in gout, cholesterol crystals in atherosclerosis, islet amyloid polypeptide, which is deposited in the pancreas in type 2 diabetes, and even simple molecules such as ATP, which is released from damaged or dying cells.

The picture is getting murkier. "Finding that the innate immune system can actually be activated by self-molecules has challenged the traditional view that the immune system only recognizes non-self as harmful," says Kate Schroder, a molecular cell biologist at the University of Queensland in Brisbane, Australia.

UNDER THE MICROSCOPE

Researchers are starting to probe the role of inflammasomes in providing defence and understanding how they distinguish harmful from harmless. One of the ways they are

building up detail is through creating images of the complex assemblages using standard confocal microscopes combined with fluorescent tagging. Confocal microscopy uses mirrors to focus light on a sample at multiple points and depths, building up a three-dimensional image. When fluorescent molecules are attached to proteins of interest — such as those that make up the inflammasome — they show up inside the cell samples.

Such fluorescent imaging is at the heart of a study published in August 2013 linking Alzheimer's disease to inflammation-causing proteins[1]. In it, researchers showed that mice lacking a molecule necessary for the activation of an inflammasome called NLRP3 did not develop the disease — suggesting a potential target for future treatments. "When you do not have inflammasomes, you do not develop any Alzheimer's, which is really cool," says Eicke Latz, one of the study's authors and director of the University of Bonn's Institute for Innate Immunity in Germany. "So you could imagine that blocking this pathway could be beneficial in humans."

Latz has also used a combination of laser reflection and fluorescence confocal microscopy to identify the way that crystalline materials interact with immune cells. His team found that feeding mice a high-fat diet caused small cholesterol crystals to appear in as little as two weeks. High levels of these crystals embedded in the vascular wall then led to activation of IL-1β (ref. 2). The researchers still do not know how the crystals activate the inflammasome, but they did identify that the complex is the trigger for the escalating response.

The emerging connection between cholesterol crystals, IL-1β and atherosclerosis — which is an inflammatory reaction that takes place in fatty blood vessel walls has prompted the first clinical trial of a drug to block IL-1β. In CANTOS, a double-blind, placebo-controlled trial run by Swiss company Novartis, 17,200 men and women who have had heart attacks are being treated with canakinumaba human monoclonal antibody that neutralizes IL-1β. The trial will follow the patients for four years and monitor whether they have any further cardiac events and other health outcomes.

Another approach looks at inflammasomes in macrophages, which are scavenger cells that consume dead cells, bacteria and viruses. Denise Monack, a microbiologist at Stanford University in California, is using confocal microscopy along with a technique called array tomography to image macrophage inflammasomes responding to bacteria such as *Salmonella* or *Fransicella*, as well as searching for genes that influence inflammasome activation.

In array tomography, Monack's group creates thin serial sections of a sample of cultured cells and stains them with fluorescent antibodies. The technique was developed by Monack's colleague Steven Smith in 2007, and Monack's lab is the only one to use it to image inflammasomes. Millions of images are snapped with a microscope and assembled into a three-dimensional structure using software. The cells can be stained with special substances packed with electrons, allowing researchers to take images at higher resolution with an electron microscope.

Imaging macrophages serially — creating two-dimensional image tiles that are reconstructed computationally into three-dimensional images — increases the resolution of the sample by an order of magnitude (from 700 nanometres down to about 70). Monack says that array tomography combines features of modern optical fluorescence and electron microscopy with better spatial resolution than confocal imaging[3], making the technique ideal for imaging the molecular architecture of an inflammasome.

"We can't explain why, but inflammasomes seem to be playing a role in so many diseases."

Monack has found that not all macrophages form inflammasomes, and that when there is an inflammasome there is only one per cell. "That's one of my burning questions: why is there only one inflammasome?" The answer, she surmises, may be that there is some kind of anchoring platform structure in the cell that allows for only one complex to form.

THE REAL THING

Dissecting this type of detail, using techniques such as array tomography or confocal microscopy, could help identify potential treatments for a range of inflammation-related conditions. "By examining the [inflammasome] structure, we may be able to design small molecule inhibitors to block either the formation of the enzymatic complex or the processing of the cytokines," says Ashley Mansell, head of the Toll-Like Receptor (TLR) Signalling Laboratory in the Centre for Innate Immunity and Infectious Diseases at the Monash Institute of Medical Research in Melbourne, Australia. One such application might be influenza: Mansell is interested in why pandemic strains drive hyper-inflammatory responses, whereas regular influenza strains do not[4].

Influenza is not the only point of interest — researchers are probing other, chronic illnesses to find targets for inflammasome inhibition. Latz points to preclinical studies that show that mice treated with an IL-1 antibody do not develop diabetes. His team is using high-throughput screening to look for other inflammasome inhibitors, but Latz thinks it will take a while to develop such a drug for clinical use. "A real inflammasome inhibitor would be nontoxic, specific and have no side effects — and that hasn't been established," he says, although he adds that the field is so new that more advances will come in the next few years.

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How are inflammasomes activated and regulated:
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A deeper understanding of inflammasomes could also be useful for diagnostics. Mansell is working on ways to get an earlier and better diagnosis of atherosclerosis or cholesterol problems with a blood test, searching for markers of inflammasome activation in the serum.

Mansell says one potential scenario is a doctor testing a patient's blood for IL-1β, and correlating that with other risk factors to offer an early warning sign. "Inflammasome activation is a marker that means there is a disruption of homeostasis," he says.

Treating or halting inflammasome activation early could stop a disease in its tracks. Mansell points to hypertension, which is a precursor to many illnesses from stroke to heart disease. Recent studies indicate a relationship between hypertension and inflammasome activation. If this link is proven, and a test developed, "we could offer people a way to treat or stop the disease from progressing further", he says.

Pharmaceutical companies are already investigating inflammasome-based therapeutics. For example, Idera Pharmaceuticals, based in Cambridge, Massachusetts, is working on treatments for psoriasis, lupus and arthritis that block toll-like receptors, which mediate the inflammasome response. And Navidea Biopharmaceuticals, based in Dublin, Ohio, has recently received approval for Lymphoseek, a lymphatic mapping agent that binds to a receptor known as CD206 on the surface of macrophages and dendritic cells — both of which are immune cells that house inflammasomes. The radiopharmaceutical binding agent allows doctors to see lymph nodes in the potential drainage path of a tumour. Originally designed for the lymph nodes around breast cancer, the agent could also point to early inflammasome involvement in some 15 diseases, says Fred Cope, Navidea's chief scientific officer.

Mansell says that this embryonic field has extensive options, because inflammasome activation is implicated in such a wide swathe of maladies. "Inflammasomes are the sparks that set things off — we can't explain why, but they seem to be playing a role in so many diseases," he says. "There have been a number of real breakthroughs in this area over the past few years." Bringing inflammasomes further into focus should yield many more. ■

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