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MEDICINE

CELLS ON FIRE

A newly discovered structure in cells underlies inflammation wherever it occurs—an insight that may lead to new treatments for ailments as diverse as atherosclerosis, Alzheimer's and fatty liver disease

By Wajahat Z. Mehal

IN BRIEF

Redness, swelling, warmth and pain have long been recognized as hallmarks of inflammation, which can be caused by infection or tissue damage.

In the past several years scientists determined that cells produce certain molecular complexes, known as inflammasomes, to launch the process.

Surprisingly, many seemingly unrelated conditions—such as Alzheimer's, gout and heart disease—share the same inflammasomes.

Investigators hope to use this insight to develop new drugs that will one day treat a wide range of chronic illnesses more effectively.

Illustration by Brian Stauffer

ANYONE WHO HAS EVER HAD A PIMPLE IS FAMILIAR WITH THE TISSUE REDNESS, swelling, warmth and pain that mark an infection. This response, known as inflammation, has been recognized since ancient times. But the process, which is often set in motion by cells of the immune system, can also occur whenever tissue is damaged—even in the absence of a pathogenic organism—as, for example, when you stub your toe or, more seriously, suffer a heart attack. This second condition is called sterile inflammation, and when it goes awry, it contributes to a wide range of seemingly unrelated medical conditions, from Alzheimer's disease to diabetes to various liver conditions.

Although prolonged inflammation and its role in disease have been known for decades, research over the past few years has yielded surprising and important insights into its origins. Among the most intriguing: inflammation is not an automatic reaction but requires the active assembly of molecular structures before it can be launched. Cells involved in inflammation build the structures—called inflammasomes—quickly and then quickly disassemble them, usually within a day of the injury. (Imagine assembling a factory in a few minutes when a product is needed and then breaking it down once the need has passed, and you get the picture.) Presumably the rapid disassembly helps the body to avoid excessive damage. Some inflammation is helpful; it kills pathogens and blocks their spread in the body. But too much can harm nearby healthy tissues and thus extend any initial injury.

Discovery of the inflammasome is interesting to biologists in its own right, but it also has profound implications for medicine. Researchers have learned that disturbances in the assembly and disassembly cycle can fuel ongoing, destructive inflammation. Right now many medicines that fight pain and swelling block the activity of certain proteins that fan the inflammatory flame. But the new work suggests that medicines able to block creation of the inflammasome or prompt its breakdown might impede the downstream production of those problematic proteins and thereby reduce tissue injury in a wholly new way. Such drugs, alone or in combination with existing ones, should help fight inflammation that currently does not respond well to therapy.

Indeed, recent discoveries about how inflammasomes sometimes go into overdrive are forcing me and other medical investigators to radically change the way we think about human disease. Rather than classifying diseases on the basis of the specific organs (heart or liver) involved, we are thinking more in terms of the cellular machinery that may be at fault: so far scientists have characterized four different versions of inflammasomes, with more likely to come. One advantage of this change in approach is that researchers can start testing whether drugs that work for, say, gout—in which one particular inflammasome is activated—may also benefit individuals with heart disease, which is triggered by the same inflammasome.

STRANGER VS. DANGER

THE INFLAMMATORY RESPONSE is part of the so-called innate branch of the immune system, typically thought of as the first line of defense against germs that invade the body. In it, white blood cells called macrophages or their relatives home to the site and then spit out proteins that induce the swelling and heat needed to immobilize and weaken microbes; the secretions also recruit still more immune cells to the area. (The pus you see in infected wounds is composed of such white cells.)

For years researchers believed that the innate system initiated this cascade solely by distinguishing "self" from "nonself." Macrophages recognize particular molecules that are common to multiple pathogens but are not present in people or other vertebrates. After making contact with these foreign molecules, the macrophages release the proteins that unleash the rest of the inflammatory response. The foreign, pathogen-only, nonself molecules are colloquially termed "stranger signals." Charles Janeway, Jr., and Ruslan M. Medzhitov, both at Yale University, laid the groundwork for this research in the late 1980s and mid-1990s.

It eventually became apparent, however, that macrophages are exquisitely reactive to certain self molecules made by the body, such as ATP (which serves as a kind of rechargeable chemical battery for cells) and the hereditary substances DNA and RNA. These molecules are usually locked securely away inside various compartments of the cell, far from the tentaclelike protrusions of any macrophages. But if self molecules spill out into the spaces between cells—which might happen when, for example, you accidentally hit your thumb with a hammer—they become detectable by proteins known as the toll-like receptors and certain other molecules on immune cells. Our body does not take a chance, and it responds to these danger signals with the assumption that strangers (pathogens) are also around; it sets off the same inflammatory response that is evoked by microbes.

This chain reaction has major consequences, the most important of which is that the inflammatory response to cellular damage can increase the amount of injury in the tissue if it fails to shut down when it is no longer needed.

GROWING EXCITEMENT

ALTHOUGH THE BROAD OUTLINE of the inflammatory response was established more than 15 years ago, excitement started building in the past decade as investigators uncovered more of the details about what exactly happens inside a macrophage before it launches such a powerful defensive reaction. Before then, investigators thought that to get to the bottom of how inflammation develops they would need to trace hundreds of molecular signals affecting dozens of different kinds of cells (including macro-

How to Light a Fire in the Body

Much to researchers' surprise, all cells in the body trigger inflammation in much the same way—by building a molecular structure, called an inflammasome, which spews out compounds called cytokines. Typically these cytokines provoke a standard, short-lived inflammatory response of redness, swelling, pain and warmth. But various ailments (such as Alzheimer's disease and gout) may result if an inflammasome remains active for too long—depending on such factors as the amount of cytokines produced and the reaction in different tissues to those cytokines. Activating substances include so-called stranger signals (produced by microbes) and danger signals (produced when the body itself becomes damaged). The discovery of a common molecular pathway may allow drug firms to develop new medications for illnesses that were previously thought to be unrelated.

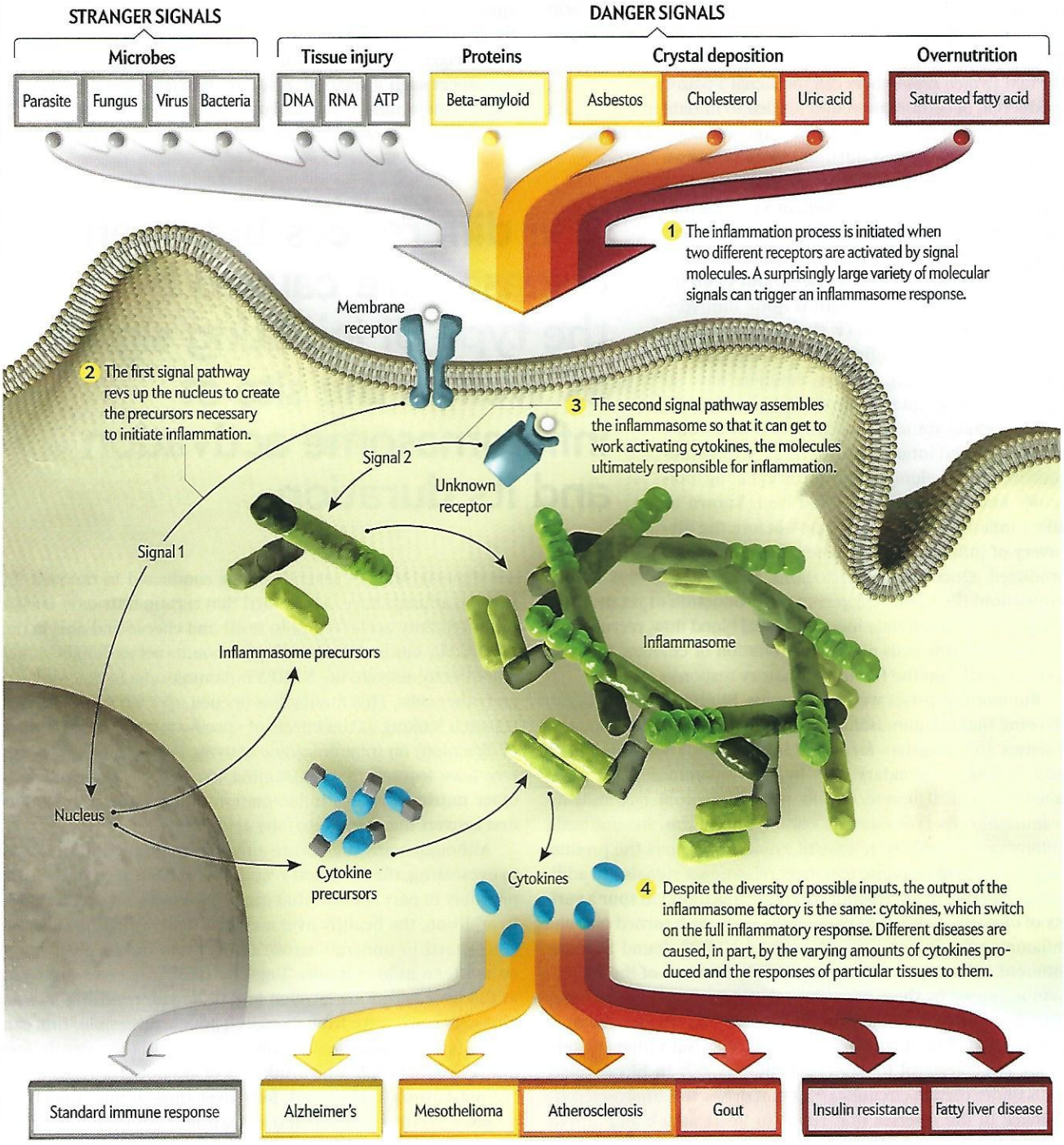

Illustration by Emily Cooper

phages). By focusing on macrophages, however, they soon realized that just a few sequences of molecular interactions, or "pathways," were needed to sound the initial alarm. Moreover, other cells used those same pathways. With just a few pathways to investigate, researchers hope to develop a handful of medications that either block the production of inflammasomes altogether or promote their disassembly in a wide variety of ailments.

So what happens inside macrophages? For starters, any macrophages near damaged cells get bathed in broken bits of DNA, RNA and other danger signals (also known as DAMPs, or danger-associated molecular patterns). Some of these danger signals bind to one particular protein on the outer surface of the macrophage cell, and others lock onto a different substance, whose identity and location are still being worked out. Once bound, these receptors activate one or the other of two different cellular processes: the first (which researchers call the signal 1 pathway) revs up the production of certain molecules needed to initiate inflammation, and the second (the signal 2 pathway) assembles an inflammasome. The fully formed inflammasome processes the newly produced inflammatory molecules in a way that activates them and then, in a process that researchers have not yet identified, releases them outside of the macrophage.

Somewhat unexpectedly, the output of an inflammasome after it gets built is quite limited—no matter whether danger signals or stranger signals get the ball rolling. Each of the four inflammatory structures that researchers have so far described ultimately produces and releases mainly two substances—specifically interleukin-1 beta (IL-1β) and interleukin-18 (IL-18). These substances, which belong to a group of signaling molecules known as cytokines, had been known to affect inflammation. But no one—before the discovery of inflammasomes—knew how they were produced. Once these interleukins are released, they spread throughout the tissue, triggering the production of yet more cytokines, which stimulate increased local blood flow, recruitment of other immune cells and a constellation of changes that collectively make up the full inflammatory response.

But more surprises were yet to come. Study after study began showing that inflammasomes are at the heart of a wide range of diseases and disorders for which inflammation was thought to play, at best, a secondary role. Indeed, inflammasomes can be constructed in all manner of cells, not just macrophages and other immune cells. (For example, certain cells in the intestine build inflammasomes whose release of cytokines triggers the production of mucus in response to danger or stranger signals.) In addition, formation of microscopic particles was found to spur a variety of diseases in different parts of the body. As it turned out, one inflammasome in particular, known as NLRP3, found in many different cells, appears to be responsible for most of the inflammation caused by these deposits—whether asbestos in the lungs (mesothelioma) or uric acid in the joints (gout). In fact, research now suggests that it is not cholesterol per se but rather the tendency of cholesterol to aggregate into crystals in blood vessel walls under certain circumstances that drives the atherosclerotic changes in the arteries that result in heart attack and stroke. Similarly in Alzheimer's, the accumulation of the protein complex beta-amyloid in the space between the neurons activates the NLRP3 inflammasome in cells known as microglia, which are the brain's equivalent of macrophages, resulting in the death of neurons. Thus, a diverse range of substances—uric acid, cholesterol, beta-amyloid, asbestos and others—results in a spectrum of diseases that affect different organs and behave in different ways but all depend on the inflammasome machinery.

The differences between diseases are caused by the type of initiating signal as well as the site of inflammasome activation and its duration.

FOOD SHOCK

THE TRUE STUNNER of the field, in my opinion, however, was the discovery that eating can trigger an inflammatory response. More specifically, eating too much in one sitting will trigger an acute episode of inflammation that eventually resolves itself, and routinely eating so many calories that the body has to store them as fat triggers chronic inflammation. Biologists had little reason to suspect such a relation. After all, nutrients are not bacteria-specific molecules or particulates, nor are they sequestered inside cells (which would make them obvious candidates for danger signals). And yet several studies conducted in the past few years in animals have determined that certain nutrients, such as saturated fatty acids (found in meat and cheese and also manufactured by our body), can in high amounts act as danger signals and directly activate the NLRP3 inflammasome in macrophages and other cells. This finding has opened up a whole new area of research looking at the effects of specific metabolites (products of digestion) on inflammasome activity. For example, investigators have learned that consuming too many carbohydrates or other nutrients causes inflammation indirectly; the body must first convert the excess into fatty acid molecules.

Although many organs are affected by inflammation related to overeating, the strongest response has been seen in the liver, probably in part because that organ takes up a lot of fatty acids. In addition, the healthy liver contains many immune cells that are primed to undergo activation and can induce liver injury even after a mild stimulus. Together these processes can result in the liver becoming swollen and inflamed, resulting in what physicians call fatty liver disease. Though reversible, this condition is often indistinguishable from what is frequently seen in the liver of people who drink a lot of alcohol. (For reasons that are not entirely understood, fatty liver disease may sometimes progress to cirrhosis—which is a potentially fatal condition.)

That finding is disturbing enough, but adding to the concern

SCIENTIFIC AMERICAN ONLINE See research pictures of inflammasomes in action at ScientificAmerican.com/jun2015/mehal

is the realization that as much as a third of obese children now have fatty liver disease. This pattern raises the possibility that at least some of them will fall ill with cirrhosis in early adulthood. It is as though large numbers of preteens were suffering from alcoholic liver disease, except the offending agent is excess calories, not alcohol. If, as animal research suggests, the NLRP3 inflammasome mediates the food-related inflammation, then it seems likely that a treatment able to prevent construction of the inflammasome could limit liver inflammation and injury in people who are overweight or obese. In support of this idea, researchers have shown that obese mice lacking inflammasome components have a healthier liver—although they are prone to infection.

Given that overnutrition can cause inflammation, my colleagues and I at Yale University decided to pursue the reverse question: whether undernutrition results in metabolites that can reduce inflammasome activation. The anti-inflammatory effects of fasting and exercise are well known, so we examined two molecules that are increased throughout the body during these states: beta-hydroxybutyrate and lactic acid. We found that the molecules interact with particular, distinct receptors on macrophages; together these interactions initiate a series of biochemical reactions in the cells that ultimately turn off the genes involved in triggering inflammasome production. Our next challenge is to figure how to harness these moderating pathways to deactivate inflammation in various diseases.

CHRONIC INFLAMMATION

THE FIRST STEP in learning how to disarm the inflammasome is to figure out how the body does it naturally—a process that normally kicks in 18 to 24 hours after an inflammasome is constructed. At the same time, researchers hope to decipher the molecular pathways that allow inflammasomes to function longer than they should during various ailments. That knowledge should suggest ways to shut down abnormally persistent inflammasomes.

For example, studies indicate that all the known danger signals—whether they trip the signal 1 or signal 2 pathway—result in a limited burst of inflammation even if the danger signals persist in the intercellular environment. After a while, immune cells simply stop responding to the long-term presence of the pathway 1 signals (the ones that rev up production) in a process called tolerance. In contrast, the pathway 2 danger signals (the ones that trigger the production of the inflammasome itself) induce the death of immune cells if they stick around too long. The result in either case is the shutting down of the inflammatory process.

Clearly then, additional signals are required to keep an inflammasome activated for a long time, as occurs in diabetes and fatty liver disease. My group, in collaboration with others, has determined that adenosine—a substance that is produced by the body whenever it breaks down ATP molecules for energy—seems to delay the dismantling of the NLRP3 inflammasome. Ironically, adenosine has long been considered an anti-inflammatory molecule because it counteracts later products of the inflammatory process.

THERAPIES TO COME

THE DISCOVERIES OUTLINED throughout this article have profoundly changed the way we view inflammation. In addition to understanding the individual steps, researchers now generally agree that many different stimuli—stranger signals, danger signals and even many of the normal breakdown products of food—converge on a single inflammatory factory (the inflammasome), which has relatively few outputs. The differences between diseases are caused by the type of the initiating signal, as well as the site of inflammasome activation and its duration. For example, uric acid crystals in joints trigger episodes of acute inflammation (gout), which resolve despite persistence of the crystals (at least until the next flare-up), but silica crystals in the lung result in chronic inflammation, followed by scarring.

This new information provides possible molecular targets against which pharmaceutical companies can try to develop new drugs. Such therapies are directed toward blocking the inflammasome at different steps in its construction, including the binding of danger signals to their receptors. Several companies have already begun experiments with different compounds that work directly on the inflammasome. But it will likely take at least a decade before these potential drug candidates can be fully tested and determined to be safe and effective.

In the meantime, many researchers have begun trying out treatments that are already effective (and have been approved by the U.S. Food and Drug Administration) for one disease on individuals with different diseases that share the same inflammasome. For example, because the drug anakinra, which has long been used to treat rheumatoid arthritis, blocks the receptor to which IL-1β binds after it leaves the inflammasome, the medication is now being tested in a wide range of NLRP3-driven diseases, including a few rare but debilitating inflammatory syndromes in children.

My group is also investigating whether the common drug digoxin, which is used to treat certain heartbeat disorders, might decrease inflammation in neurological disorders such as Alzheimer's. Other researchers recently demonstrated that digoxin inhibits a molecule called HIF-1α. My group at Yale then determined that HIF-1α is required for sustained activation of the NLRP3 inflammasome. Because NLRP3 appears to be active in the brain of Alzheimer's patients, our combined results suggest that digoxin might be a potential Alzheimer's treatment—although much further study is needed. Too much digoxin has been shown to cause confusion and other symptoms that mimic dementia, and it can have other side effects as well.

The past few years have seen an explosion of research into the basic biology of the inflammasome. The next few years will produce insights and possibly new therapies in ways that cannot be entirely predicted. But the rich and complex organization of this astonishing cellular factory makes it clear that tackling inflammation at its source could relieve more of the suffering and disability that currently makes life so difficult for so many people. SA

MORE TO EXPLORE

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