INITIAL ENCOUNTER with germs, or pathogens, sets off the "innate" arm of the immune system, which turns out to be more sophisticated than anyone guessed.

Immunity's Early-Warning System

By Luke A. J. O'Neill

The innate immune response constitutes the first line of defense against invading microbes and plays a role in inflammatory disease. Surprising insights into how this system operates could lead to new therapies for a host of infectious and immune-related disorders

A woman is riding an elevator when her fellow passengers start to sneeze. As she wonders what sort of sickness the other riders might be spreading, her immune system swings into action. If the bug being dispersed by the contagious sneezers is one the woman has met before, a battalion of trained immune cells—the foot soldiers of the so-called adaptive immune system—will remember the specific invader and clear it within hours. She might never realize she had been infected.

But if the virus or bacterium is one that our hapless rider has never wrestled, a different sort of immune response comes to the rescue. This "innate" immune system recognizes generic classes of molecules produced by a variety of disease-causing agents, or pathogens. When such foreign molecules are detected, the innate system triggers an inflammatory response, in which certain cells of the immune system attempt to wall off the invader and halt its spread. The activity of these cells—and of the chemicals they secrete—precipitates the redness and swelling at sites of injury and accounts for the fever, body aches and other flulike symptoms that accompany many infections.

The inflammatory assault, we now know, is initiated by Toll-like receptors (TLRs): an ancient family of proteins that mediate innate immunity in organisms from horseshoe crabs to humans. If TLRs fail, the entire immune system crashes, leaving the body wide open to infection. If they work too hard, however, they can induce disorders marked by chronic, harmful inflammation, such as arthritis, lupus and even cardiovascular disease.

Discovery of TLRs has generated an excitement among immunologists akin to that seen when Christopher Columbus returned from the New World. Scores of researchers are now setting sail to this new land, where they hope to find explanations for many still mysterious aspects of immunity, infection and disorders involving abnormal defensive activity. Study of these receptors, and of the molecular events that unfold after they encounter a pathogen, is already beginning to uncover targets for pharmaceuticals that may enhance the body's protective activity, bolster vaccines, and treat a range of devastating and potentially deadly disorders.

Cinderella Immunity

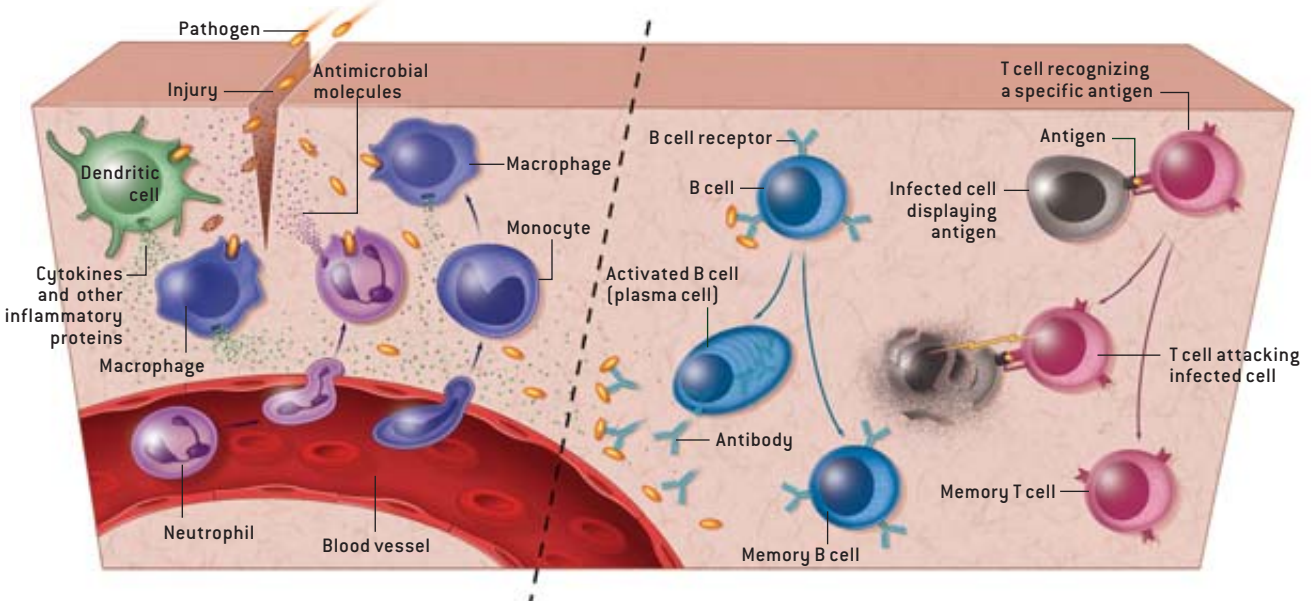
UNTIL ABOUT FIVE YEARS AGO, when it came to the immune system, the adaptive division was the star of the show. Textbooks were filled with details about B cells making antibodies that latch onto specific proteins, or antigens, on the surface of an invading pathogen and about T cells that sport receptors able to recognize fragments of proteins from pathogens. The response is called adaptive because over the course of an infection, it adjusts to optimally handle the particular microorganism responsible for the disease.

Adaptive immunity also grabbed the spotlight because it endows the immune system with memory. Once an infection has been eliminated, the specially trained B and T cells stick around, priming the body to ward off subsequent attacks. This ability to remember past infections allows vaccines to protect us from diseases caused by viruses or bacteria. Vac-

JAMES SALZANO

THE DIVISIONS OF THE IMMUNE SYSTEM

The mammalian immune system has two overarching divisions. The innate part (*left side*) acts near entry points into the body and is always at the ready. If it fails to contain a pathogen, the adaptive division (*right side*) kicks in, mounting a later but highly targeted attack against the specific invader.



INNATE IMMUNE SYSTEM

This system includes, among other components, antimicrobial molecules and various phagocytes (cells that ingest and destroy pathogens). These cells, such as dendritic cells and macrophages, also activate an inflammatory response, secreting proteins called cytokines that trigger an influx of defensive cells from the blood. Among the recruits are more phagocytes—notably monocytes (which can mature into macrophages) and neutrophils.

ADAPTIVE IMMUNE SYSTEM

This system "stars" B cells and T cells. Activated B cells secrete antibody molecules that bind to antigens—specific components unique to a given invader—and destroy the invader directly or mark it for attack by others. T cells recognize antigens displayed on cells. Some T cells help to activate B cells and other T cells (*not shown*); other T cells directly attack infected cells. T and B cells spawn "memory" cells that promptly eliminate invaders encountered before.

cines expose the body to a disabled form of a pathogen (or harmless pieces of it), but the immune system reacts as it would to a true assault, generating protective memory cells in the process. Thanks to T and B cells, once an organism has encountered a microbe and survived, it becomes exempt from being overtaken by the same bug again.

The innate immune system seemed rather drab in comparison. Its components—including antibacterial enzymes in saliva and an interlocking set of proteins (known collectively as the complement) that kill bacteria in the bloodstream—were felt to be less sophisticated than targeted antibodies and killer T cells. What is more, the innate immune system does not tailor its response in the same way that the adaptive system does.

In dismissing the innate immune response as dull and uninteresting, however, immunologists were tiptoeing around a dirty little secret: the adaptive system does not work in the absence of the allegedly more crude innate response. The innate system produces certain signaling proteins called cytokines that not only induce inflammation but also activate the B and T cells that are needed for the adaptive response. The posh sister, it turns out, needs her less respected sibling to make her shine.

By the late 1990s immunologists knew a tremendous amount about how the adaptive immune system operates. But they had less of a handle on innate immunity. In particular, researchers did not understand how microbes activate the innate response—or exactly how this stimulation helps to drive the adaptive response of T and B cells. Soon after, though, they would learn that much of the answer lay with the TLRs, which are produced by various immune system cells. But the path scientists traveled to get to these proteins was a circuitous one,

Overview/*Innate Immunity*

- Innate immunity serves as a rapid response system for detecting and clearing infections by any infectious agent. The response is mediated by a family of molecules called Toll-like receptors (TLRs), made by many defensive cells.
- When TLRs detect an invader, they trigger the production of an array of signaling proteins that induce inflammation and direct the body to mount a full-fledged immune response.
- If TLRs are underactive, the immune system fails; if overactive, they can give rise to disorders such as rheumatoid arthritis and even cardiovascular disease. Learning how to manipulate TLRs or the proteins with which they interact could provide new options for treating infectious and inflammatory diseases.

TAMI TOLPA

winding through studies of fruit fly development, the search for drugs to treat arthritis, and the dawn of the genomic era.

Weird Protein

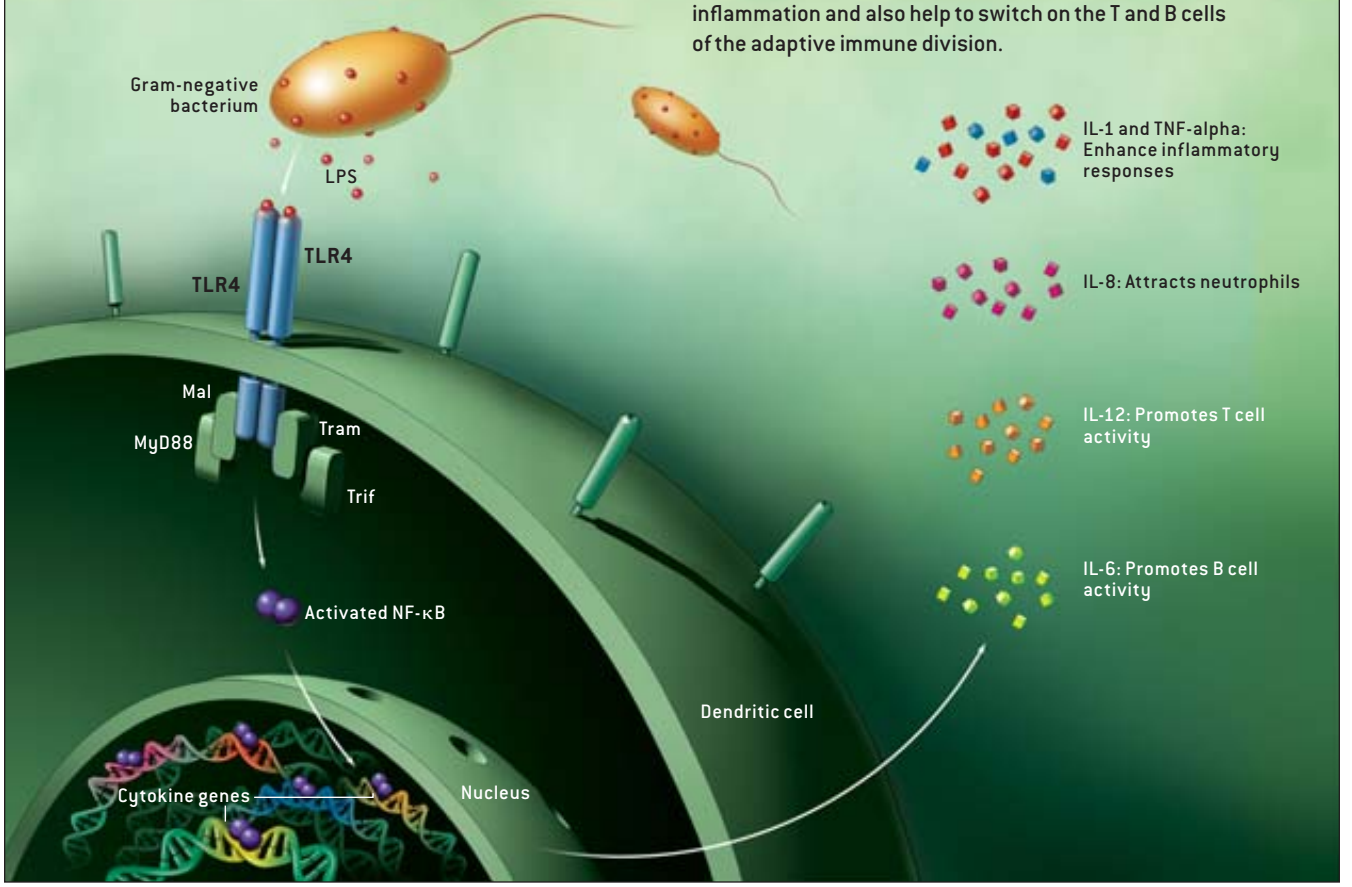
THE PATH ACTUALLY HAD its beginnings in the early 1980s, when immunologists started to study the molecular activity of cytokines. These protein messengers are produced by various immune cells, including macrophages and dendritic cells. Macrophages patrol the body's tissues, searching for signs of infection. When they detect a foreign protein, they set off the inflammatory response. In particular, they engulf and destroy the invader bearing that protein and secrete a suite of cytokines, some of which raise an alarm that recruits other cells to the site of infection and puts the immune system in general on full alert. Dendritic cells ingest invading microbes and head off to the lymph nodes, where they present fragments of the pathogen's proteins to armies of T cells and release cytokines—activities that help to switch on the adaptive immune response.

To study the functions of various cytokines, researchers needed a way to induce the molecules' production. They found that the most effective way to get macrophages and dendritic cells to make cytokines in the laboratory was to expose them to bacteria—or more important, to selected components of bacteria. Notably, a molecule called lipopolysaccharide (LPS), made by a large class of bacteria, stimulates a powerful immune response. In humans, exposure to LPS causes fever and can lead to septic shock—a deadly vascular shutdown triggered by an overwhelming, destructive action of immune cells. LPS, it turns out, evokes this inflammatory response by prompting macrophages and dendritic cells to release the cytokines tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1).

Indeed, these two cytokines were shown to rule the inflammatory response, prodding immune cells into action. If left unchecked, they can precipitate disorders such as rheumatoid arthritis, an autoimmune condition characterized by excessive inflammation that leads to destruc-

TOLLS IN CHARGE

Toll-like receptors (TLRs), made by many cells of the innate system, have been found to both orchestrate the innate immune response and play a critical role in the adaptive response. TLR4, for example, elicits these defenses when gram-negative bacteria begin to invade. TLR4 detects the incursions by binding to lipopolysaccharide (LPS), a sugar unique to gram-negative bacteria. Having recognized LPS, pairs of TLR4s signal to four molecules inside the cell—MyD88, Mal, Tram and Trif—which, in turn, trigger molecular interactions that ultimately activate a master regulator of inflammation (NF-κB). This regulator then switches on genes that encode immune activators, including cytokines. These cytokines (*far right*) induce inflammation and also help to switch on the T and B cells of the adaptive immune division.



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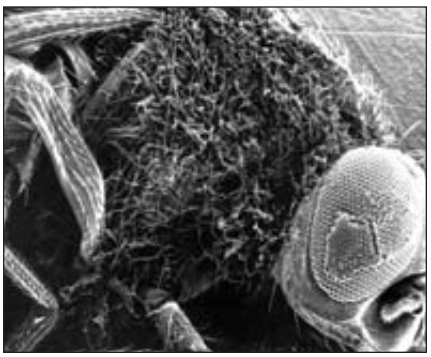
tion of the joints. Investigators therefore surmised that limiting the effects of TNF-alpha and IL-1 might slow the progress of the disease and alleviate the suffering of those with arthritis. To design such a therapy, though, they needed to know more about how these molecules work. And the first step was identifying the proteins with which they interact.

In 1988 John E. Sims and his colleagues at Immunex in Seattle discovered a receptor protein that recognizes IL-1. This receptor resides in the membranes of many different cells in the body, including macrophages and dendritic cells. The part of the receptor that juts out of the cell binds to IL-1, whereas the segment that lies inside the cell relays the message that IL-1 has been detected. Sims examined the inner part of the IL-1 receptor carefully, hoping it would yield some clue as to how the protein transmits its message—revealing, for example, which signaling molecules it activates within cells. But the inner domain of the human IL-1 receptor was unlike anything researchers had seen before, so he was stymied.

Then, in 1991, Nick J. Gay of the University of Cambridge—working on a completely unrelated problem—made a strange discovery. He was looking for proteins that were similar to a fruit-fly protein called Toll. Toll had been identified by Christiane Nusslein-Volhard in Tübingen, Germany, who gave the protein its name because flies that lack Toll look weird (*Toll* being the German word for "weird"). The protein helps the developing *Drosophila* embryo to differentiate its top from its bottom, and flies without Toll look jumbled, as if they have lost their sidedness.

Gay searched the database containing all the gene sequences then known. He



FRUIT FLY that lacked the protein Toll fell victim to a rampant fungal infection; spores cover the body like a fur coat. (The head is at the bottom right.) This outcome, reported in 1996, was one of the first indications that fruit flies require Toll proteins for protection against disease.

was looking for genes whose sequences closely matched that of Toll and thus might encode Toll-like proteins. And he discovered that part of the Toll protein bears a striking resemblance to the inner part of the human IL-1 receptor, the segment that had mystified Sims.

At first the finding didn't make sense. Why would a protein involved in human inflammation look like a protein that tells fly embryos which end is up? The discovery remained puzzling until 1996, when Jules A. Hoffmann and his collaborators at CNRS in Strasbourg showed that flies use their Toll protein to defend themselves from fungal infection. In *Drosophila*, it seems, Toll multitasks and is involved in both embryonic development and adult immunity.

Worms, Water Fleas and You

THE IL-1 RECEPTOR and the Toll protein are similar only in the segments that are tucked inside the cell; the bits that are exposed to the outside look quite different. This observation led researchers to search for human proteins that resemble Toll in its entirety. After all, evolution usually conserves designs that work well—and if Toll could mediate immunity in flies, perhaps similar proteins were doing the same in humans.

Acting on a tip from Hoffmann, in 1997 Ruslan Medzhitov and the late Charles A. Janeway, Jr., of Yale University discovered the first of these proteins, which they called human Toll. Within six months or so, Fernando Bazan and his colleagues at DNAX in Palo Alto, Calif., had identified five human Tolls, which they dubbed Toll-like receptors (TLRs). One, TLR4, was the same human Toll described by Medzhitov and Janeway.

At that point, researchers still did not know exactly how TLRs might contribute to human immunity. Janeway had found that stuffing the membranes of dendritic cells with TLR4 prompted the production of cytokines. But he could not say how TLR4 became activated during an infection.

The answer came in late 1998, when Bruce Beutler and his co-workers at the Scripps Institute in La Jolla, Calif., found that mutant mice unable to respond to LPS harbor a defective version of TLR4. Whereas normal mice die of sepsis within an hour of being injected with LPS, these mutant mice survive and behave as if they have not been exposed to the molecule at all; that is, the mutation in the TLR4 gene renders these mice insensitive to LPS.

This discovery made it clear that TLR4 becomes activated when it interacts with LPS. Indeed, its job is to sense LPS. That realization was a major breakthrough in the field of sepsis, because it revealed the molecular mechanism that underlies inflammation and provided a possible new target for treatment of a disorder that sorely needed effective therapies. Within two years, researchers determined that most TLRs—of which 10 are now known in humans—recognize molecules important to the survival of bacteria, viruses, fungi and parasites. TLR2 binds to lipoteichoic acid, a component of the bacterial cell wall. TLR3 recognizes the genetic material of viruses; TLR5 recognizes flagellin, a protein that forms the whiplike tails used by bacteria to swim; and TLR9 recognizes a signature genetic sequence called CpG, which occurs in bacteria and viruses in longer stretches and in a form that is chemically distinct from the CpG sequences in mammalian DNA.

TLRs, it is evident, evolved to recognize and respond to molecules that are fundamental components of pathogens. Eliminating or chemically altering any one of these elements could cripple an infectious agent, which means that the or-

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FROM BRUNO LEMAITRE, EMMANUELLE NICOLAS, LYDIA MICHAUT, JEAN-MARC REICHHART AND JULES A. HOFFMANN IN *CELL*, VOL. 86, NO. 6, PAGES 973–983; SEPTEMBER 20, 1996

ganisms cannot dodge TLRs by mutating until these components are unrecognizable. And because so many of these elements are shared by a variety of microbes, even as few as 10 TLRs can protect us from virtually every known pathogen.

Innate immunity is not unique to humans. In fact, the system is quite ancient. Flies have an innate immune response, as do starfish, water fleas and almost every organism that has been examined thus far. And many use TLRs as a trigger. The nematode worm has one that allows it to sense and swim away from infectious bacteria. And plants are rife with TLRs. Tobacco has one called N protein that is required for fighting tobacco mosaic virus. The weed *Arabidopsis* has more than 200. The first Toll-like protein most likely arose in a single-celled organism that was a common ancestor of plants and animals. Perhaps these molecules even helped to facilitate our evolution. Without an efficient means of defense against infection, multicellular organisms might never have survived.

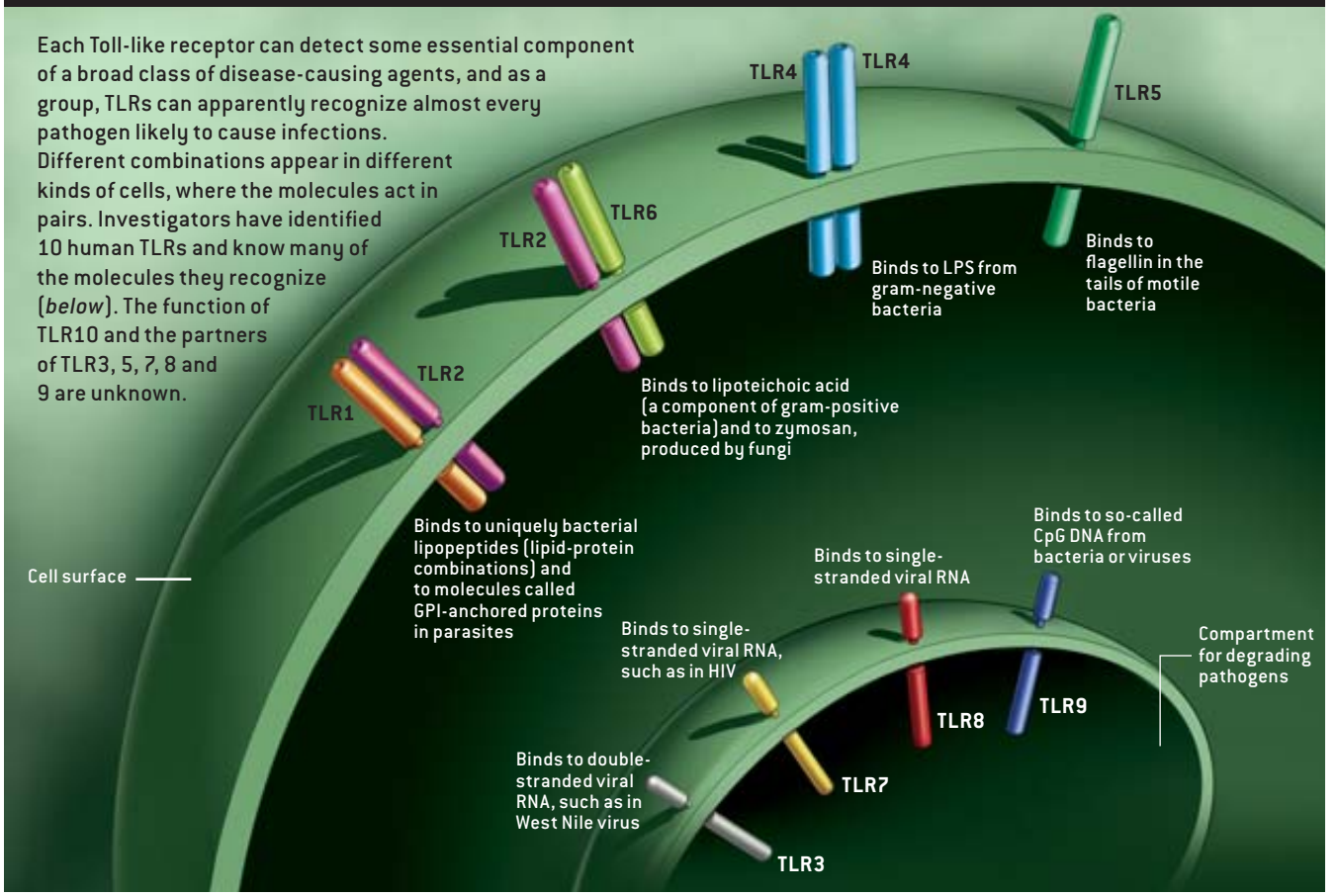
Storming the Castle

THE INNATE SYSTEM was once thought to be no more elaborate than the wall of a castle. The real action, researchers believed, occurred once the wall had been breached and the troops inside—the T and B cells—became engaged. We now know that the castle wall is studded with sentries—TLRs—that identify the invader and sound the alarm to mobilize the troops and prepare the array of defenses needed to fully combat the attack. TLRs, in other words, unleash both the innate and adaptive systems.

The emerging picture looks something like this. When a pathogen first enters the body, one or more TLRs, such as those on the surface of patrolling macrophages and dendritic cells, latch onto the foreign molecules—for example, the LPS of gram-negative bacteria. Once engaged, the TLRs prompt the cells to unleash particular suites of cytokines. These protein messengers then recruit additional macrophages, dendritic cells and other immune cells to wall off and nonspecifically attack the marauding microbe. At the same time, cytokines released by all these busy cells can produce the classic symptoms of infection, including fever and flulike feelings.

Macrophages and dendritic cells that have chopped up a pathogen display pieces of it on their surface, along with other molecules indicating that a disease-causing agent is present. This display, combined with the cytokines released in response to TLRs, ultimately activates B and T cells that recognize those specific pieces, causing them—over the course of several days—to proliferate and launch a powerful, highly focused attack on the particular invader. Without the priming effect of TLRs, B and T cells would not become engaged and the body would not

THE JOBS OF TOLL-LIKE RECEPTORS



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be able to mount a full immune response. Nor could the body retain any memory of previous infections.

Following the initial infection, enough memory T and B cells are left behind so that the body can deal more efficiently with the invader should it return. This army of memory cells can act so quickly that inflammation might not occur at all. Hence, the victim does not feel as ill and might not even notice the infection when it recurs.

Innate and adaptive immunity are thus part of the same system for recognizing and eliminating microbes. The interplay between these two systems is what makes our overall immune system so strong.

Choose Your Weapon

TO FULLY UNDERSTAND how TLRs control immune activity, immunologists need to identify the molecules that relay signals from activated TLRs on the cell surface to the nucleus, switching on genes that encode cytokines and other immune activators. Many investigators are now pursuing this search intensively, but already we have made some fascinating discoveries.

We now know that TLRs, like many receptors that reside on the cell surface, enlist the help of a long line of signaling proteins that carry their message to the nucleus, much as a bucket brigade shuttles water to a fire. All the TLRs, with the exception of TLR3, hand off their signal to an adapter protein called MyD88. Which other proteins participate in the relay varies with the TLR: my laboratory studies Mal, a protein we discovered that helps to carry signals generated by TLR4 and TLR2. TLR4 also requires two other proteins—Tram and Trif—to relay the signal, whereas TLR3 relies on Trif alone. Shizuo Akira of Osaka University in Japan has shown that mice engineered so that they do not produce some of these intermediary signaling proteins do not respond to microbial products, suggesting that TLR-associated proteins could provide novel targets for new anti-inflammatory or antimicrobial agents.

Interaction with different sets of signaling proteins allows TLRs to activate different sets of genes that hone the cell's response to better match the type of pathogen being encountered. For example, TLR3 and TLR7 sense the presence of viruses. They then trigger a string of molecular interactions that induce the production and release of interferon, the major antiviral cytokine. TLR2, which is activated by bacteria, stimulates the release of a blend of cytokines that does not include interferon but is more suited to activating an effective antibacterial response by the body.

The realization that TLRs can detect different microbial products and help to tailor the immune response to thwart the enemy is now overturning long-held assumptions that innate immunity is a static, undiscriminating barrier. It is, in fact, a dynamic system that governs almost every aspect of inflammation and immunity.

From *Legionella* to Lupus

ON RECOGNIZING the central role that TLRs play in initiating immune responses, investigators quickly began to suspect that hobbled or overactive versions of these receptors could contribute to many infectious and immune-related disorders. That hunch proved correct. Defects in innate immunity lead to greater susceptibility to viruses and bacteria. People with an underactive form of TLR4 are five times as likely to have severe bacterial infections over a five-year period than those with a normal TLR4. And people who die from Legionnaire's disease often harbor a mutation in TLR5 that disables the protein, compromising their innate immune response and rendering them unable to fight off the *Legionella* bacterium. On the other hand, an overzealous immune response can be equally destructive. In the U.S. and Europe alone, more than 400,000 people die annually from sepsis, which stems from an overactive immune response led by TLR4.

Other studies are pointing to roles for TLRs in autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. Here TLRs might respond to products from damaged cells, propagating an inappropriate inflammatory response and promoting a misguided reaction by the adaptive immune system. In lupus, for example, TLR9 has been found to react to the body's own DNA.

Innate immunity and the TLRs could also play a part in heart disease. People with a mutation in TLR4 appear to be less prone to developing cardiovascular disease. Shutting down TLR4 could protect the heart because inflammation appears to contribute to the formation of the plaques that clog coronary arteries.

MECHNIKOV'S FLEAS

The discovery of Tolls and Toll-like receptors extends a line of research begun more than 100 years ago, when Russian biologist Ilya Mechnikov essentially discovered innate immunity. In the early 1880s Mechnikov plucked some thorns from a tangerine tree and poked them into a starfish larva. The next morning he saw that the thorns were surrounded by mobile cells, which he surmised were in the process of engulfing bacteria introduced along with the foreign bodies. He then discovered that water fleas (*Daphnia*) exposed to fungal spores mount a similar response. This process of phagocytosis is a cornerstone of innate immunity, and its discovery earned Mechnikov a Nobel Prize in 1908.

MECHNIKOV was a character. Speaking of the era when he worked at the Pasteur Institute, his Nobel Prize biography notes, "It is said of him that at this time he usually wore overshoes in all weathers and carried an umbrella, his pockets being overfull with scientific papers, and that he always wore the same hat, and often, when he was excited, sat on it."

HULTON ARCHIVE/GETTY IMAGES

TLRs AS DRUG TARGETS

Agents that activate TLRs and thus enhance immune responses could increase the effectiveness of vaccines or protect against infection. They might even prod the immune system to destroy tumors. In contrast, drugs that block TLR activity might prove useful for dampening inflammatory disorders. Drugs of both types are under study (*below*).

DRUG TYPE	EXAMPLES
TLR4 activator	MPL, an allergy treatment and vaccine adjuvant (immune system activator) from Corixa (Seattle), is in large-scale clinical trials
TLR7 activator	ANA245 (isatoribine), an antiviral agent from Anandys (San Diego), is in early human trials for hepatitis C
TLR7 and TLR8 activator	Imiquimod, a treatment for genital warts, basal cell skin cancer and actinic keratosis from 3M (St. Paul, Minn.), is on the market
TLR9 activator	ProMune, a vaccine adjuvant and treatment for melanoma skin cancer and non-Hodgkin's lymphoma from Coley (Wellesley, Mass.), is in large-scale clinical trials
TLR4 inhibitor	E5564, an antisepsis drug from Eisai (Teaneck, N.J.), is in early human trials
General TLR inhibitor	RDP58, a drug for ulcerative colitis and Crohn's disease from Genzyme (Cambridge, Mass.), is entering large-scale clinical trials
General TLR inhibitor	OPN201, a drug for autoimmune disorders from Opsona Therapeutics (Dublin, Ireland), is being tested in animal models of inflammation

Manipulation of TLR4 might therefore be another approach to preventing or limiting this condition.

Volume Control

MANY OF THE BIG pharmaceutical companies have an interest in using TLRs and their associated signaling proteins as targets for drugs that could treat infections and immune-related disorders. With the spread of antibiotic resistance, the emergence of new and more virulent viruses, and the rising threat of bioterrorism, the need to come up with fresh ways to help our bodies fight infection is becoming more pressing.

Work on TLRs could, for example, guide the development of safer, more effective vaccines. Most vaccines depend on the inclusion of an adjuvant, a substance that kick-starts the inflammatory response, which in turn pumps up the ability of the adaptive system to generate the desired memory cells. The adjuvant used in most vaccines today does not provoke a full adaptive response; instead it favors B cells over T cells. To elicit a stronger response, several companies have set their sights on compounds that activate TLR9, a receptor that recognizes a broad range of bacteria and viruses and drives a robust immune response.

And TLRs are teaching us how to defend ourselves against biological weapons, such as poxviruses. A potential staple in the bioterrorist arsenal, these viruses can shut down TLRs and thereby avoid detection and elimination. In collaboration with Geoffrey L. Smith of Imperial College London, my lab found that by removing the viral protein that disables TLRs, we could generate a weakened virus that could serve as the basis of a vaccine unlikely to provoke an unintended fatal pox infection.

Armed with an understanding of TLRs and innate immunity, physicians might be able to predict which patients will fare poorly during infection and treat them more aggressively. If, for instance, patients came to a clinic with a bacterial infection and were found to have a mutant TLR4, the doctor might bombard them with antibiotics or with agents that could somehow bolster their immune response to prevent the infection from doing lasting damage.

Of course, a balance must be struck between stimulating an immune response that is sufficient to clear a microbe and precipitating an inflammatory response that will do more harm than good. Similarly, any medications that aim to relieve inflammation by quelling TLR activity and cytokine release must not, at the same time, undercut the body's defense against infection.

Anti-inflammatory drugs that interfere with TNF-alpha, one of the cytokines produced as a result of TLR4 activation, offer a cautionary tale. TNF-alpha produced during infection and inflammation can accumulate in the joints of patients with rheumatoid arthritis. The anti-inflammatory compounds alleviate the arthritis, but some people taking them wind up with tuberculosis. The infection is probably latent, but reining in the inflammatory response can also dampen the pathogen-specific responses and allow the bacterium to reemerge.

In short, TLRs are like the volume knob on a stereo, balancing adaptive immunity and inflammation. Researchers and pharmaceutical companies are now looking for ways to tweak these controls, so they can curtail inflammation without disabling immunity.

Given that TLRs were unheard of seven years ago, investigators have made enormous progress in understanding the central role these proteins play in the body's first line of defense. Innate immunity, long shrouded in oblivion, has suddenly become the belle of the ball. SA

MORE TO EXPLORE

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