Biologists reveal the proteins that first see dangerous microbes

By JOHN TRAVIS

About 3 weeks after a 4-year-old boy visited the emergency room for a nagging eye infection—which doctors easily cured—the boy's mother arrived at the same Canadian hospital. She was infected with the same bacterium, though it had done much more than redden her eyes. She showed signs of shock: plummeting blood pressure and a racing heart.

Physicians suspected sepsis, the destructive overreaction of the immune system to an overwhelming bacterial infection of the blood. They gave the woman massive doses of antibiotics, but it was too late. Just hours after arriving at the hospital, she died.

Both mother and son had been infected with *Neisseria meningitides*. Why had the boy beat the bacterium but his mother had succumbed? No one knows for sure, but the answer could lie in the two having had differences in certain proteins that stud the surfaces of white blood cells. These so-called toll-like receptors, or TLRs, are the key sensors guiding the body's initial reaction to bacteria, viruses, or fungi. Scientists call this first line of defense the innate immune response.

Over the past 4 years, scientists have found that TLRs respond to microbial features such as a bacterium's cell wall, tail, or even its DNA. In doing so, the receptors trigger white blood cells to engulf and kill infectious microbes or to signal other immune cells to rally to the cause.

Toll-like receptors are the "eyes of the innate immune system," according to Bruce Beutler of the Scripps Research Institute in La Jolla, Calif. "These receptors are the main way that the innate immune system sees pathogens. That had been a big mystery for the last 100 years or so," he says.

The recent discovery of the human versions of these immune sensors has ignited a research frenzy. "The number of papers that have been published on toll-like receptors in the last 2 years is astounding. It's hard to keep up," says Steven R. Kleeberger of Johns Hopkins University School of Public Health in Baltimore.

Insights from TLRs could help immunologists fight sepsis or design vaccines that ward off infections in the first place. Someday, profiling a person's TLR genes "could tell who's at risk to get sepsis," suggests Beutler. Moreover, drugs that block TLRs may stop the immune overreaction responsible for sepsis or thwart other disorders of the immune system.

The field is "moving at the speed of light," says Fabio Re of Dana-Farber Cancer Institute in Boston. To scientists' surprise, they have even found that TLRs show up on fat cells (see box, page 153), may play a role in premature births, and may contribute to lung damage from air pollution.

About a century ago, Russian biologist Elie Metchnikoff offered the first glimpse at the innate immune system when he reported that a thorn stuck in a starfish was rapidly surrounded by amoeba-like cells. In people, white blood cells called macrophages, neutrophils, eosinophils, and dendritic cells lead this front line of immunity.

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The innate immune system depends upon white blood cells called macrophages (balls) and dendritic cells (sail shapes).

While these soldiers of the innate immune system can sometimes fend off invading microbes on their own, they often simply hold the pathogens in check until the adaptive, or acquired, immune system comes to the rescue. This later phase of the immune response depends upon so-called T and B cells.

Traditionally, immunology researchers have focused on adaptive immunity, a bias that Douglas T. Fearon of the University of Cambridge in England noted several years ago. "Despite its evolutionary success, innate immunity has been treated with condescension by immunologists. It has been considered a stopgap measure, a temporary expedient for host defense, buying time until acquired immunity took over," he remarked. "In short, innate immunity was unsophisticated, unintelligent, indiscreet, and obsolescent."

With the discovery of TLRs in the 1990s, that viewpoint has itself become obsolete, Fearon and other immunologists argue. They contend that the innate system not only calls forth the adaptive system but provides it with chemical cues that tailor the response of T and B cells. "It's absolutely clear that the triggering of the innate immune system is a required first step in the recruitment of cells of adaptive immunity and in the training of those cells to see things," says David M. Underhill of the University of Washington in Seattle.

The original receptor named Toll was a fly protein first found to have a role in development, not immunity. Then, scientists observed that flies with a mutation in the protein's gene were unable to fight off fungi. Further investigation revealed that the fly protein recognizes a separate insect protein produced during a fungal infection and in turn, activates the fly's immune response.

In 1997, a group led by Charles A. Janeway Jr., a Howard Hughes Medical Institute investigator at Yale University, identified the human version of this receptor. Its activation in a human immune cell triggers the synthesis of molecules called cytokines, which contribute to inflammation, his team found. Moreover, the stimulated immune cell produces another protein that activates quiescent T cells. In short, this work suggested that TLRs link the innate and adaptive arms of the human immune system.

Despite their recent discovery, the receptors appear to have a long history, notes Robert Modlin of the University of California, Los Angeles. Related molecules help plants fight off infections, so TLR ancestors were probably present hundreds of millions of years ago, before plants and animals evolved into separate kingdoms, he explains.

...iologists following up on Janeway's ...ork soon discovered that the human ...enome contains at least 10 genes encod... TLRs. Investigators quickly confirmed ...that TLRs in mammals help innate im... cells recognize pathogens.

...Beutler's group, for example, studied ...mutant mice that don't respond to ...lipopolysaccharide, or LPS, a cell wall ...component of certain bacteria. The mice ...are more susceptible to infection by LPS-...bearing microbes than other mice are. In ...1998, the scientists discovered that their ...LPS-tolerant mice had mutations in the ...mouse gene that corresponds to *TLR4*, ...the human gene originally identified by ...Janeway and his colleagues. LPS is also ...known as endotoxin because it's impor... in sepsis. When the mammalian ...body reacts to LPS, the immune system ...goes into overdrive, releasing a flood of ...cytokines that produce fever, shock, and ...often death.

"We realized that toll-like receptor 4 was the endotoxin receptor," says Beutler.

Immunologists now conclude that TLR4 and at least one other protein form a complex on immune cells that binds to LPS and goads the cells into action.

Different TLRs seem to recognize distinct microbial features. For example, TLR2 was once thought to recognize LPS also, but recent studies have linked it instead to other molecules made by a variety of bacteria, including the ones that cause tuberculosis. And in the April 26 SCIENCE, Underhill and his colleagues presented evidence that TLR5 detects flagellin, a protein found only in the whip-like tails of many bacteria.

Last year, a research team led by Shizuo Akira of Osaka University in Japan reported that TLR9 helps the human immune system recognize bacterial DNA. Scientists have sought to exploit this unusual capability by creating vaccines made of pure DNA (SN: 12/4/99, p. 385), but such vaccines tend to work better in mice than in people.

Offering an explanation, a German research team reported in the July 31 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES that the two species have slightly dissimilar TLR9s and recognize different bacterial DNA sequences.

CDC

In a process called phagocytosis, this mouse cell engulfs a bacterium. Toll-like receptors may help such cells recognize microbes.

Immune receptors offer new view of fat cells

Scientists are rethinking the fat cell, and the newly discovered toll-like receptors may play an integral part in that reevaluation.

Until recently, biologists considered fat cells relatively uninteresting. "For decades, they were pretty much viewed as fat blobs, as inert storage compartments for triglycerides," says Philipp Scherer of the Albert Einstein College of Medicine in New York.

That worldview shifted in 1994, when scientists discovered that fat cells secrete leptin, a hormone that travels to the brain and regulates feeding (SN: 12/3/94, p. 372). All of a sudden, fat was seen as an endocrine organ.

Now, says Scherer, there's growing evidence that fat cells can do many of the things that immune cells so. For example, they can release and respond to the chemicals called cytokines, which regulate the immune system, particularly the inflammatory reaction.

Last year, Scherer's group reported that fat cells make TLR4. Moreover, when the researchers exposed fat cells to lipopolysaccharide (LPS), one of the bacterial components that activates TLR4, the cells began to make a related receptor, TLR2, and to synthesize secreted immune molecules such as interleukin-6.

Scherer plans to test whether LPS-activated fat cells produce small, pathogen-killing proteins called antimicrobial peptides. He's also curious whether fat cells can swallow and kill microbes as the immune cells called macrophages do. "We basically view the fat cell as an extremely fat macrophage," he says. —*J.T.*

...orting out what microbial features a white blood cell's TLR detects will take some time. The complexity of the situation has grown with the discovery that different TLRs may work together.

Last December, Underhill and his colleagues demonstrated that TLR2 can pair with TLR6 to recognize different bacterial proteins from those that TLR2 alone detects. If such pairings are common among the 10 or so known human TLRs, combinations of the receptors may be able to distinguish a far greater variety of microbial parts than single receptors do.

"That changes the thinking [about TLRs] a great deal," says Underhill.

A looming controversy for those working on TLRs is whether the proteins respond to products of the mammalian body itself. Several research groups have reported TLR reactions to products of mammalian cells: molecules called heat-shock proteins and specific fragments of the blood-clotting protein fibronectin.

Why would the receptors react to such products? One explanation may lie in the controversial "danger hypothesis" put forth by Polly Matzinger, an immunologist at the National Institutes of Health in Bethesda, Md. She challenges the traditional notion that the immune system distinguishes between self and nonself and responds directly to the latter. She argues, instead, that the immune system reacts to microbes only when danger signals have been released by infected or injured cells.

Damaged cells discharge heat shock proteins and fibronectin, providing a possible connection between TLRs and Matzinger's ideas. "Besides sensing danger from the outside, they may see danger signals from the self," says Modlin.

Other researchers say it's difficult to establish that TLRs recognize mammalian molecules. "I don't think the evidence is good enough yet," says Beutler.

...hatever molecules induce TLRs to action, researchers are striving to understand what happens next in the immune response. In the Feb. 23 SCIENCE, for example, Modlin and his colleagues showed that activating one TLR leads directly to the death of a microbe. They reported that triggering TLR2 on macrophages infected with the tuberculosis bacterium prompts the immune cells to destroy the pathogens .

Of perhaps greater interest is the link that TLRs provide between the innate and adaptive immune systems. In the simplest scenario, the innate immune cells could send up a generalized distress call for T and B cells. There are hints of greater sophistication, however.

In a paper recently published online by the JOURNAL OF BIOLOGICAL CHEMISTRY, Re and his colleague Jack L. Strominger report that dendritic cells of the innate immune system behave differently depending on whether their TLR2s or TLR4s are triggered. Activate TLR2 on the white blood cell's surface and it releases one broth of immune molecules; stimulate TLR4 and the dendritic cell secretes a different soup.

Re calls the dendritic cell the "orchestral conductor" of adaptive immunity, noting that the chemicals secreted by it shape the manner in which T and B cells act. Consequently, dendritic cells, through the activation of their various TLRs, may inform the adaptive immune system what type of pathogen has invaded and guide the body's overall response.

Figuring out the roles of all the TLRs and learning how to stimulate them artificially may open up new ways to combat infections and improve vaccines. "We will be able to tailor a specific immune response," suggests Re.

On the other hand, in diseases such as sepsis, in which the immune response is the problem, activating TLRs is the last

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thing physicians would want. Indeed, a promising sepsis drug now being tested in people turns out to block TLR4 from reacting to the LPS in a bacterium's cell wall. "There may be some situations where we want to activate tolls and others where we want to block tolls," says Modlin.

Another area of growing interest is explorations of how people's TLR genes vary. Changes in those genes may help explain why certain people or populations are more or less vulnerable to specific microbes. According to preliminary evidence from Beutler's group, people with mutations in one TLR are more susceptible to the bacterial infections that can cause sepsis than other people are.

"Collectively, the toll-like receptors have a strong influence on the course of infections. They may be the major determinants of susceptibility to many infectious diseases," says Beutler.

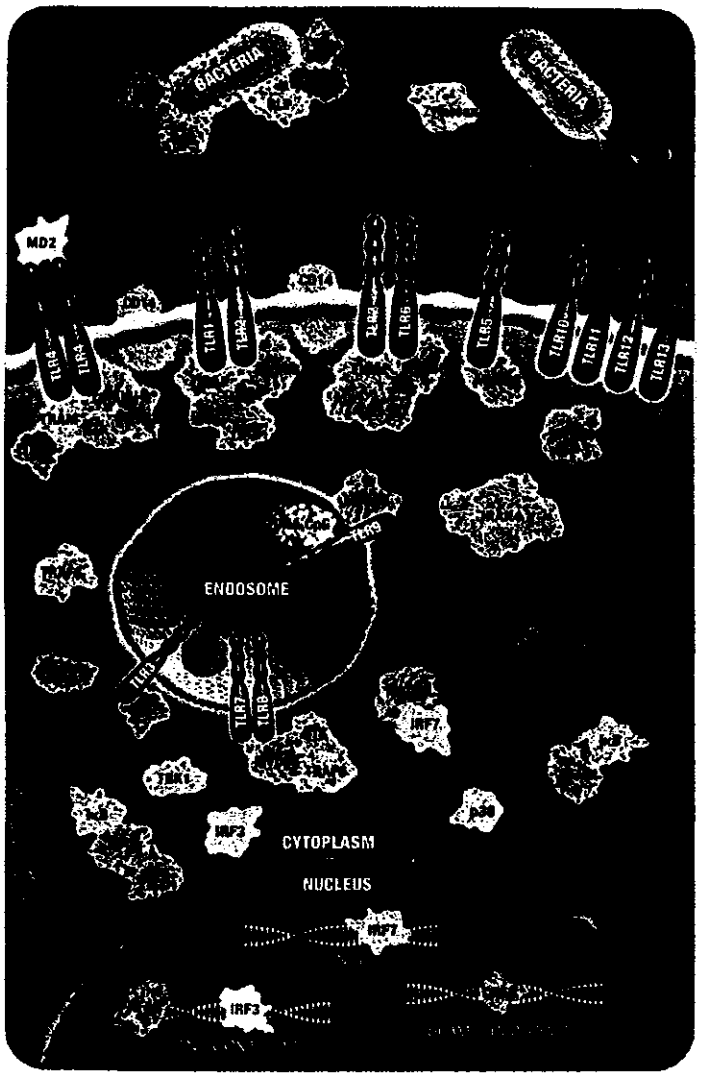
Lately, scientists other than immunologists have started paying attention to toll-like receptors. Kleeberger's work focuses on how ozone and other forms of air pollution damage lungs and why some people are more susceptible than others. His team unexpectedly detected a possible role for TLR4 in that damage. When exposed to ozone, mice lacking TLR4 suffer less lung damage than normal mice do, the group reported in the February AMERICAN JOURNAL OF PHYSIOLOGY.

Kleeberger speculates that ozone initially injures cells in the lungs, inducing the release of molecules that activate TLR4 and create more damage through an immune reaction. His group now plans to study whether variations in the TLR4 gene govern a person's susceptibility to ozone.

The course of pregnancy may also be regulated by TLRs, says Jerome F. Strauss III of the University of Pennsylvania Medical Center in Philadelphia whose group reported last March that certain fragments of the clotting protein fibronectin activate TLR4. That's provocative because data by other teams connect increased concentrations of the fragments in a pregnant woman's blood to a higher risk of premature birth. Also, some infections have been associated with preterm births, adding to suspicion that TLR activation influences the timing of birth. "We're looking for better markers to identify women at risk of preterm birth," says Strauss.

Still, it's the immunologists who are most thrilled with their discoveries about TLRs. According to Evelyn Kurt-Jones of the University of Massachusetts in Amherst, who studies how these receptors may recognize viruses, the recent research "has provided some exciting new ways to look at the innate immune system. It has refocused interest on the earliest events in the immune response." □

Toll-like Receptors and Innate Immunity



Toll-like Receptor Signaling Pathway

Innate and Adaptive Immunity

The immune system is divided into two parts, the innate and adaptive systems. The adaptive immune response depends on B and T lymphocytes which are specific for particular antigens. This system involves clonal selection of antibody producing B cells to respond to foreign antigens, and works well, but has a major limitation in that it takes from 4 to 7 days to ramp up. In that time period, pathogens could overwhelm the organism.

In contrast, the innate immune system is immediately available to combat threats. There is no complicated method of selecting cells that react to foreign substances from those that react to self. There is no memory to change how the system responds to the same threat upon the second or third exposure. Instead, the innate immune system responds to common structures shared by a vast majority of threats. These common structures are called pathogen associated molecular patterns, or PAMPs, and are recognized by the toll-like receptors, or TLRs. In addition to the cellular TLRs, an important part of the innate immune system is the humoral complement system that opsonizes and kills pathogens through the PAMP recognition mechanism.

These highly conserved soluble and membrane bound proteins are collectively called Pattern-Recognition Receptors (PRRs), and it is the PAMP/PRR interaction that triggers the innate immune system. While the history of TLR-dependent observations goes back 100 years, most of the definitive work started about fifteen years ago. A tremendous amount of work has been done during this time, including the discovery of other PRR pathways. The cytosolic NOD (nucleotide oligomerization domain) proteins have been shown to be important innate immune response components.

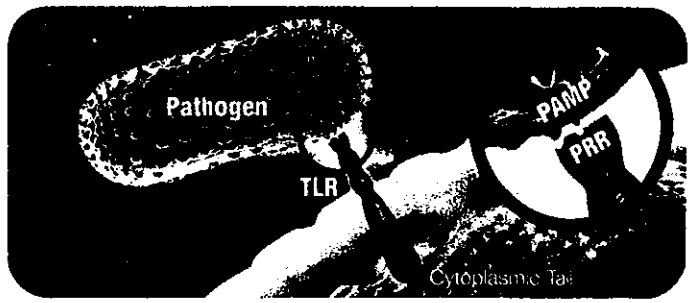


Figure 1: Binding of a Pathogen via its PAMP (Pathogen Associated Molecular Pattern) to a TLR's PRR (Pattern Recognition Receptor) Domain. The Extracellular Leucine-Rich Repeats of the TLR, constitute the PRR Region.

Toll-like Receptors (TLR)

TLRs are transmembrane proteins expressed by cells of the innate immune system, which recognize invading microbes and activate signaling pathways that launch immune and inflammatory responses to destroy the invaders. Toll receptors were first identified in Drosophila. In mammals, the TLR family includes eleven proteins (TLR1–TLR11). Recently, two new members, TLR12 and TLR13 have been discovered in murine cells, but not much information is known about them. Mammalian TLRs consist of an extracellular portion containing leucine-rich repeats, a transmembrane region and a cytoplasmic tail, called the TIR (Toll-IL-1R (Interleukin-1-Receptor)) homology domain. Different TLRs serve as receptors for diverse ligands, including bacterial cell wall components, viral double-stranded RNA and small-molecule anti-viral or immunomodulatory compounds (Table 1).

Activation of TLRs occurs after binding of the cognate ligand to the extracellular leucine-rich repeats portion of the TLR. In humans, TLR1, 2,4,5 and 6 are outer membrane associated, and respond primarily to bacterial surface associated PAMPs. The second group, TLR3,7,8 and 9 are found on the surface of endosomes, where they respond primarily to nucleic acid based PAMPs from viruses and bacteria. Upon binding with their cognates, TLRs activate two major signaling pathways. The core pathway utilized by most TLRs leads to activation of the transcription factor NF-κB (Nuclear Factor-κB) and the MAPKs (Mitogen-Activated Protein Kinases) p38 and JNK (c-Jun N-termal Kinase).

The second pathway involves TLR3 and TLR4 and leads to activation of both NF-κB and another transcription factor IRF3 (Interferon Regulatory Factor-3), allowing for an additional set of genes to be induced, including anti-viral genes such as IFN-β (Interferon-Beta) and others (1). The innate immune response is a complex set of interactions that have evolved to optimize the response to pathogens. While the structure of the TLRs has been highly conserved, the innate immune response for each organism has selectively been driven to best protect against the pathogens found in the host's environment.

Table 1: The TLRs and Their Pathogen Derived Activators (2).

PAMP	PRR	Pathogen
Pam3CSK4, PGN, Zymosan et al	TLR1,2,6	Gram Positive Bacteria
LPS, Lipid A	TLR4	Gram Negative Bacteria
Flagellin	TLR5	Bacteria Flagellum
dsRNA	TLR3	Virus
ssRNA	TLR7,8	Virus
CpG DNA	TLR9	Bacteria, DNA

Innate Defense Against Bacteria

As shown in Table 1, the defense against bacteria involves all the TLRs except for 3,7 and 8 which are virus specific. Gram positive and negative bacteria differ in their surface PAMPs and bind to different TLRs. The specificity of the TLRs for bacterial cell wall fragments has just recently been established, as early reports were later found to be due to trace contaminants.

The signal transduction pathway for TLR4 activation by LPS (Lipopolysaccharide) serves as a representative example of the surface bound TLRs (see the opening illustration of the pathway).

LPS first binds to the CD14 (Cluster of Differentiation-14) receptor, which then transfers it to TLR4. TLR4 homodimerizes and forms a complex with the protein MD2. Cells need both MD2 and TLR4 in order to recognize LPS. TLR4 activation engages a set of MyD88 (Myeloid Differentiation Primary-Response Protein-88) adaptor family members, including TIRAP, TRIF, TRAM (all three are TIR domains containing adapter proteins) and MyD88. This pattern of activation is general for cell surface TLRs, but the subsequent intracellular signal cascades, which include a number of transcription factor activations, are unique for each TLR. This results in a response that is appropriate to each threat (1).

TLR2 is activated by bacterial LAM (Lipoarabinomannan), BLP (Bacterial Lipoprotein), and PGN (Peptidoglycans). LAM and PGN act on TLR2 through the CD14 receptor, similar to the process followed by the TLR4 with a similar downstream effect. BLP mediates both apoptosis and NF-κB activation through TLR2. TLR2 is also responsible for the recognition of the Yeast cell-wall particle Zymosan. Zymosan acts through the CD14 receptor to influence TLR2. The phagocytosed TLR2 vessicle signals the production of TNF (Tumor Necrosis Factor), through the NF-κB pathway. TLR6 associates with TLR2 and recognizes diacylated MALP2 (Mycoplasmal macrophage-Activating Lipopeptide-2 kD). Like TLR4, they also signal through MyD88 and TIRAP. PI3K (Phosphatidylinositide-3 Kinase), RIP2 (Receptor-Interacting Protein-2) and Rac (Ras-Related C3 Botulinum Toxin Substrate) are also involved in TLR6-TLR2 mediated signaling. TLR1 also associates with TLR2 and recognizes the native mycobacterial 19-kDa lipoprotein along with TLR2. TLR1-TLR2 also signals through MyD88, TIRAP, PI3K, RIP2 and Rac. TLR1 and TLR6 may participate in the activation of macrophages by gram positive bacteria. TLR5 is a signaling mediator of bacterial flagellin, thus activating NF-κB and may play a role in resistance to Salmonella infection (3). Human TLR10 is an orphan member of the TLR family. Genomic studies indicate that TLR10 is in a locus that also contains TLR1 and TLR6, two receptors known to function as coreceptors for TLR2. TLR10 not only homodimerizes but also heterodimerizes with TLRs 1 and 2. It has been found to activate gene transcription through MyD88. TLR9 is responsible for the recognition of CpG islands of bacterial DNA. The extracellular CpG fragment may activate TLR9, thus inducing the endocytosis of the DNA along with TLR9, or perhaps the bacteria is phagocytosed and TLR9, which has separately formed on the phagosome, is activated by the CpG islands; whatever the exact method, TLR9 activates the NF-κB pathway from the endocytosed vesicle. Recently IRF8 (Interferon Regulatory Factor-8) has been shown to be activated by TLR9 through MyD88 (3).

Co-receptors on TLR-bearing cells play a critical role in the inflammatory response. In monocytes for example, the CD36 and CD14 co-receptors are necessary for the TLR2 response to gram positive bacteria.

The continued arms race between bacteria and immune defense mechanisms is demonstrated by pyloric bacteria, which have evolved a modified flagellum that evades detection by TLR5, helping this pathogen to establish residency in the mammalian digestive system.

Figure 2: The Flagella of H. pylori *(Helicobacter pylori)* Does Not Activate TLR5 due to Sequence Changes in the Flagellar Protein That Prevent Detection by TLR5.

In addition to the TLRs, two NOD (nucleotide oligomerization domain) proteins in the cytoplasm, have recently been found to play an important role in the innate defense against E. coli & S. aureus. The NOD proteins contain leucine-rich repeats very similar to those in TLRs that recognize specific components of these bacteria (diaminopimelic acid) and form a cytoplasmic signaling platform with other proteins known as the inflammasome. This signaling leads to IL-1 & IL-8 production.

Recently it has been argued that while it is well established that a strong innate defense response to bacteria is essential for survival, the most important role of this TLR activation in the long term, may be in the induction of the adaptive immune response. This is discussed further in the three sentinal cells section.

Innate Defense Against Viruses

Viral nucleic acids contain PAMPs that are recognized by intracellular TLRs. These TLRs are located on the intracellular endosome membranes. The TLRs found on endosomes are TLR3, TLR7, TLR8 and TLR9. TLR3 activates immune cells in response to double-stranded viral RNA. The stimulation of the TLR3 triggers TRIF activation that ultimately activates the IRF3 transcription factor through TBK1, independent of MyD88. This leads to the secretion of IFN-β. TRIF also activates RIP1 (Receptor-Interacting Protein-1) and TRAF6, which may further activate the NF-κB pathway. Small anti-viral compounds activate immune cells via the TLR7/MyD88-dependent signaling pathway. TLR7 binds MyD88 and activates IRAF and TRAF6. TRAF6 then activates TANK (also known as I-TRAF). TANK interacts with TBK1 and IKK-ε to activate IRF3. TLR7 or TLR8 may also activate IRF7 through interaction of MyD88, BTK and TRAF6, thus inducing anti-viral responses by producing IFN-α (Interferon-Alpha). Recently, Mouse TLR11 has been identified as a participant in defense against uropathogenic bacteria. The ligands for Mouse TLR12 and TLR13 are currently unknown.

It should be noted that only plasmacytoid cells use the TLR pathway for viral defense. Other cells use RIG1 (retinoic acid inducible gene1)-like helicases (RLHs) to recognize viral PAMPs which results in primarily an IFN response (4,5). The fact that plasma cells utilize TLRs suggests that the TLR-dependent response to viral infection is both important for immediate viral protection, as well as the activation of adaptive immunity via the inflammatory cytokines. A number of studies have shown that a weakened TLR response to particular viruses leads to poor antibody & Th1 responses, and the combination leads to persistent viral infections.

Innate Defense Against Parasites

The study of TLR activation in parasitic diseases is just beginning, but these early results indicate a significant role. Polymorphisms in TLRs have been linked with the severity of systemic malarial infections. In contrast, an intact TLR signaling system has been shown to contribute to the severe cerebral malarial infection that is often lethal (6). This is an example of how a vigorous TLR response to a parasite can lead to a more severe disease.

In leishmaniasis, which affects 10 million people, TLR2 and TLR4 are required for proper parasite control, due to the activity of inducible nitric oxide (iNOS). A second factor induced by TLR4 activation, neutrophil elastase, is also important for the leishmanicidal activity of macrophages (7).

Toxoplasma gondii, the common parasite causing toxoplasmosis in humans, binds to the newly discovered TLR11, which has no other known ligands in humans.

Figure 3: TLR11 Recognizes and Is Activated by the T. Gondii Parasite.

The Three Sentinal Cells of Innate Immunity and TLRs

TLRs are primarily found on macrophages, mast cells and dendritic cells, the three sentinal cells of the innate immune response. It is interesting that the surface expression of TLRs in humans is highly variable and this has been linked to susceptibility to infections (8). It should be noted that the activation of TLRs on these cells begins a complex set of signaling cascades that are not yet completely understood. These interactions are not restricted to the innate immune response, but they also play an important role in adaptive immunity.

We are at an exciting point in immunological research, where our knowledge of the innate immune response, and how it guides adaptive immunity, may lead to more effective treatments of immunological diseases.

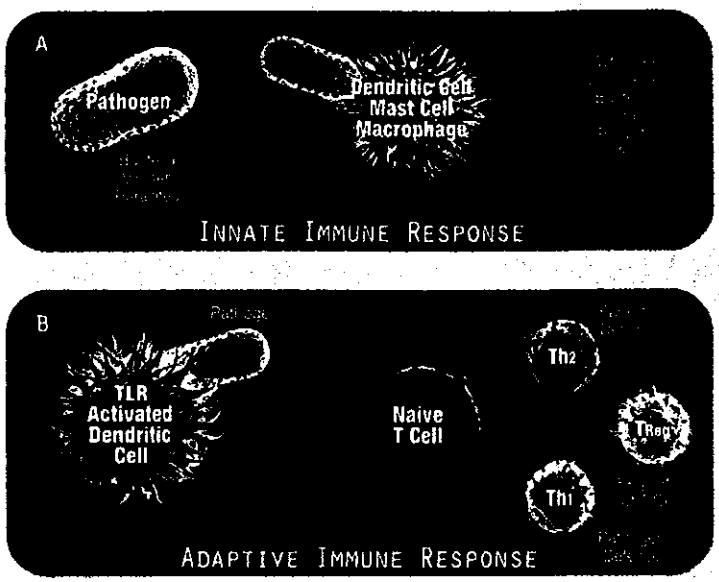


Figure 4: The 3 Sentinal Cells, Dendritic, Mast, & Macrophages Protect Against Pathogens. Dendritic Cells Also Are Critical in the Adaptive Immune Response.

Fine Tuning TLR Activation

Unchecked TLR activation by pathogens can lead to serious medical consequences, such as sepsis and autoimmune diseases. In the last few years, negative modulators of TLR activation have been identified, and their important role in reducing the inflammatory response has been demonstrated in animal models (9-11). The TAM family members are one example.

The TAM family, has been found to be central to the fine tuning of the TLR response. Loss of function of the three members of this family (Tyro3/Axl/Mer) in a triple knockout mouse results in a profound dysregulation of the immune response (10). This includes massive splenomegaly and lymphadenopathy, lymphocyte infiltration into all tissues, and high levels of autoimmunity. Even a single knockout of just Mer is sufficient to elevate susceptibility to LPS induced shock via the TLR4 signaling. These mice have elevated levels of dendritic cells, and the cells express elevated levels of activation markers, including MHC class II antigens. This effect was not restricted to TLR4, as hypersensitivity to the TLR3 activator polyIC was also observed. While the details of the mechanisms for this modulation of the innate immune response are not yet known, the TAM receptor ligands Gas6 and ProS, play an important role. The inhibitory effect requires the synthesis of SOCS1 (suppressor of cytokine signaling 1) which had been previously identified in the cytokine response. Further downstream, the transcription of STAT1 was shown to be essential, as was the IFN receptor IFNAR1.

Summary

Innate immunity is recognized to play an important role in the response to challenge by pathogens.

The immune functions in which toll-like receptors play important roles include:

- Orchestration of the immediate tissue specific and global response of the innate immune system to pathogens. This orchestration is driven primarily by cytokine and chemokine production (TNF, Interferons, IL-1, IL-2, IL-6, IL-8 and IL-12 among others). Perhaps the most important of these early signals are the chemokines that draw the phagocytes to the site of infection.

- Transition from innate to adaptive immunity. In addition to the role in the innate immune response, TLRs have an important role in adaptive immunity by activating antigen presenting cells. The cytokine signaling cascade stimulated by TLR activation, begins a complex series of interactions that has evolved in each organism to maximize the odds for survival. Among the more important of these signals is T cell differentiation and regulation. TLRs on dendritic cells in particular, are essential in the T-helper-1 (Th1) versus Th2 pathways (12). An important early component of the Th1 response is the activation of cytotoxic T cells that helps to control the infection (Fig 4).

Our knowledge of the complex innate immune response is rapidly increasing. An organism's survival depends on a prompt response to pathogens, but it is equally important to avoid unregulated inflammation that can lead to dangerous pathologies such as sepsis and autoimmune disease. It is this fine balance between protection and self-damage that drives the complexity of the innate immune response.

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SABiosciences Toll-Like Receptor Research Products

SABiosciences offers a number of research tools for the study of toll-like receptors and their signaling pathways:

Technology	Product	Catalog #
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PCR Array	Interferons (IFN) & Receptors PCR Array	Human PAHS-064 Mouse PAMM-064
PCR Array	Chemokines & Receptors PCR Array	Human PAHS-022 Mouse PAMM-022 Rat PARN-022
PCR Array	Inflammatory Cytokines PCR Array	Human PAHS-011 Mouse PAMM-011 Rat PARN-011
Oligo Microarray	Inflammatory Cytokines & Receptors GEArray	Human OHS-011 Mouse OMM-011 Rat ORN-011 2
siRNA Array	NF-κB Signaling Pathway siRNA Array	Human SAH-025A
ELISArray	Th1, Th2, Th17 Cytokines Multi-Analyte Kits	Human MEH-003A Mouse MEM-003A
ELISArray	Common Cytokines	Human MEH-006A Mouse MEM-006A
ELISArray	Inflammatory Cytokines	Human MEH-004A Mouse MEM-004A
Cell-Based Assay	Cignal NF-κB Reporter Assay Kit (luciferase)	Human CCS-013L
Cell Based Assay	Cignal ISRE Reporter Assay Kit (luciferase)	Human CCS-008L
Cell Based Assay	Cignal GAS Reporter Assay Kit (luciferase)	Human CCS-009L
Cell Based Assay	Cignal SMAD Reporter Assay Kit (luciferase)	Human CCS-017L
Cell-Based Assay	Cignal NF-κB Reporter Assay Kit (GFP)	Human CCS-013G
Cell Based Assay	Cignal SMAD Reporter Assay Kit (GFP)	Human CCS-017G
Cell Based Assay Array	Cignal Finder Immune Response 10-Pathway Reporter Array (luc)	Human CCA-002L
Cell-Based Assay Array	Cignal Finder Immune Response 10-Pathway Reporter Array (plate)	Human CCA-102L

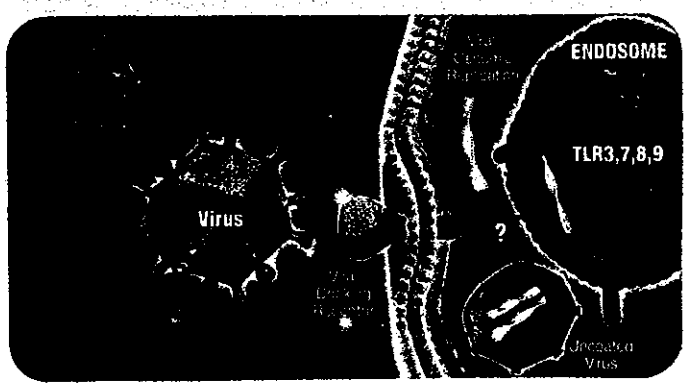

Figure 5: Viral Nucleic Acids Activate Endosomal TLR3,7,8,9.