Pattern recognition receptors in innate immunity, host defense, and immunopathology

Rahul Suresh and David M. Mosser

Department of Cell Biology and Molecular Genetics and the Maryland Pathogen Research Institute, University of Maryland, College Park, Maryland

Submitted 18 June 2013; accepted in final form 2 August 2013

Suresh R, Mosser DM. Pattern recognition receptors in innate immunity, host defense, and immunopathology. Adv Physiol Educ 37: 284–291, 2013; doi:10.1152/advan.00058.2013.—Infection by pathogenic microbes initiates a set of complex interactions between the pathogen and the host mediated by pattern recognition receptors. Innate immune responses play direct roles in host defense during the early stages of infection, and they also exert a profound influence on the generation of the adaptive immune responses that ensue. An improved understanding of the pattern recognition receptors that mediate innate responses and their downstream effects after receptor ligation has the potential to lead to new ways to improve vaccines and prevent autoimmunity. This review focuses on the control of innate immune activation and the role that innate immune receptors play in helping to maintain tissue homeostasis.

pattern recognition receptors; Toll-like receptors; pathogen-associated molecular patterns; damage-associated molecular patterns; chemokines; antigen presentation; vaccines

THE FIELD OF IMMUNOLOGY over the past two decades has gradually shifted its focus from antigen-specific recombining receptors (on T and B cells) to a set of germline encoded receptors that play important roles in innate immunity. These receptors are involved in not only identifying nonself microbial products but also “danger” signals from the host that foretell of changes in homeostasis. These receptors have been termed pattern recognition receptors (PRRs), which serve to identify pathogen-associated molecular patterns (PAMPs) (27) and danger-associated molecular patterns (DAMPs) (37). Janeway (28) suggested that these PRRs were important for the initiation of adaptive immunity. The seminal work that identified the first PRR was provided by Le Maître and Hoffman (34), when they demonstrated that mutant flies lacking a functional Toll protein were susceptible to fungal infections. This initiated a rush of studies to identify PRRs and to understand how innate immunity could initiate and shape adaptive immune responses. Since then, many surface and cytosolic PRRs have been identified in higher mammals and throughout evolution, and we are beginning to understand how signaling through these receptors allows for the initiation of adaptive immune responses. We have also come to realize that the activation of these PRRs can lead to inflammatory immunopathology, and therefore studies to understand how PRR signaling can be modified or interrupted are providing new hope for the development of therapeutics to treat inflammatory diseases.

There are two major consequences of ligation of PRRs on immune cells. The first is to signal danger to the cells and to initiate a cascade of responses that direct host defense responses. Most of these responses are in the form of cytokines or antimicrobial compounds that are produced by leukocytes in response to PRR stimulation. The second important consequence is to induce the competency of select cells to present antigen to T cells. The presentation of antigen by antigen-presenting cells (APCs) is necessary to initiate adaptive immune responses. PRR stimulation induces dendritic cell (DC) maturation to stabilize major histocompatibility complex (MHC) molecules on their surface, and it facilitates antigen presentation by inducing the expression of costimulatory molecules on APCs to induce T cell proliferation and differentiation. Thus, the ligation of PRRs can benefit the host in two ways. First, it can initiate innate immune responses to directly kill pathogens, and, if those responses are not sufficient to clear the pathogen, it can initiate antigen-specific adaptive immune responses to provide a second layer of protection.

Toll-Like Receptors in Innate Immunity:

The Toll-like receptor (TLR) family of receptors consists of membrane-bound PRRs responsible for detecting various PAMPs. These PAMPs are generally structures that are indispensable for the survival of the pathogen and are, hence, generally conserved. The relative lack of ligand variability allows innate immune cells with germline-encoded TLRs to recognize these molecular patterns and initiate signaling responses. Despite the fact that each of the TLRs recognizes different molecular patterns, the structure of all TLRs is generally quite conserved. All TLRs contain an extracellular leucine-rich repeat (LRR) domain that is responsible for ligand binding. The coiled structure of LRRs allows for a flexible backbone upon which the ligand-binding motifs are imposed. The LRR domain is connected to a transmembrane domain and an intracellular Toll/IL-1 receptor (TIR) domain that is responsible for signal transduction. When bound to a ligand, TLRs form homodimers or heterodimers and initiate signaling pathways that are very similar to the IL-1 receptor-mediated pathway of NF-κB activation (60). TLR signaling is initiated when the cytoplasmic TIR domain interacts with adaptor molecules such as myeloid differentiation primary response gene 88 (MyD88). Other TLR adapters include TIR domain-containing adaptor protein, TIR domain-containing adaptor inducing interferon (IFN)-β (TRIF), MyD88 adaptor-like, TIR domain-containing adaptor molecule-1, and TRIF-related adaptor molecule (31). MyD88 is the adaptor molecule that is used by all TLRs except TLR3. The binding of MyD88 to TLRs leads to the activation of IL-1 receptor-associated kinases by MyD88,
which causes downstream activation of a complex containing TNF receptor-associated factor (TRAF)6 and transforming growth factor (TGF)-β-activated kinase 1-binding protein 2 (Fig. 1). This activates TGF-β-activated kinase (TAK)-1. TAK-1 then serves as a branch point, leading to the activation of both NF-κB and MAPK signaling pathways (Fig. 1) (38). The types of adaptor molecules used for signaling and the strength of signaling may influence the class and extent of transcription factors that are activated downstream of the TLRs. TLR1, TLR2, TLR4, TLR5, and TLR6 are found on the cell surface and typically recognize ligands that are usually bacterial in origin. TLR3, TLR7, TLR8, and TLR9 are responsible for recognizing nucleic acids including double-stranded (ds)RNA and CpG DNA. Nucleic acids of host origin with TLR-activating motifs can be found in cells, so these TLRs are not exclusive for pathogen nucleic acids. Nonself ligands are selectively identified by their presence in endosomal compartments where the TLRs are usually expressed. Self nucleic acids are normally sequestered from these compartments to prevent TLR ligation. There are two important exceptions to the original idea that TLRs recognize only pathogen-associated (nonself) antigens. The first exception is the recognition of DAMPs that are expressed on endogenous “alarmins” released from damaged and dying cells (44), and the second exception is the inappropriate localization of nucleic acids that can occur during some pathological conditions.

Improving Vaccines by Incorporating TLR Activators as Adjuvants

Adjuvants can boost the magnitude and duration of the adaptive immune response to a particular antigen. One of the ways adjuvants act is through the activation of PRRs on innate immune cells. An important consequence of PRR activation is the maturation of DCs. DCs are APCs involved in presenting antigens to naïve T cells. Upon TLR activation, there is increased expression of factors on the surface of DCs required for antigen presentation, such as MHC class II and accessory signals like CD40 and CD80/86. TLR-stimulated DCs also secrete cytokines that can influence the type of adaptive immune response that develops. For example, IL-12 is a cytokine produced by DCs after TLR stimulation. IL-12 is important in promoting the Th helper cell (Th1) immune response that is vital for the control of intracellular pathogens. As shown in Fig. 2, mice were vaccinated with a fusion protein consisting of four Mycobacterium tuberculosis antigens, called ID93 (3). The adjuvant contained the TLR4 agonist glucopyranosyl lipid adjuvant (GLA) induced protective immunity. Guinea pigs were injected with saline or were immunized with bacille Calmette-Guerin (BCG) as a positive control, ID93/SE (stable oil in water emulsion adjuvant without GLA), or ID93/GLA-SE. The survival of guinea pigs after M. tuberculosis infection was measured up to day 210. Saline-injected and ID93/SE-immunized guinea pigs succumbed to M. tuberculosis infection, whereas those immunized with BCG or ID93/GLA-SE survived. [Reprinted with permission from Ref. 3.]

Fig. 1. The Toll-like receptor (TLR) signaling pathway. TLR ligation leads to the activation of NF-κB and MAPKs. Myeloid differentiation primary response gene 88 (MyD88), a key TLR adapter protein, associates with IL-1 receptor-associated kinase (IRAK)4, which, in turn, phosphorylates IRAK1. After its interaction with TNF receptor-associated factor (TRA)6, the activated IRAK complex phosphorylates transforming growth factor-β-activated kinase 1/MAP3K-binding protein 1 (TAB1) and TGF-β-activated kinase-1, which activate NF-κB and MAPK pathways. MD-2, myeloid differentiation protein-2; IKK, IκB kinase. [Reprinted with permission from Ref. 38.]

Fig. 2. Vaccination against Mycobacterium tuberculosis. Immunization with M. tuberculosis antigen ID93 along with TLR4 agonist glucopyranosyl lipid adjuvant (GLA) induced protective immunity. Guinea pigs were injected with saline or were immunized with bacille Calmette-Guerin (BCG) as a positive control, ID93/SE (stable oil in water emulsion adjuvant without GLA), or ID93/GLA-SE. The survival of guinea pigs after M. tuberculosis infection was measured up to day 210. Saline-injected and ID93/SE-immunized guinea pigs succumbed to M. tuberculosis infection, whereas those immunized with BCG or ID93/GLA-SE survived. [Reprinted with permission from Ref. 3.]
suggest that a deeper understanding of TLRs and the various ways to target antigen to them could lead to the generation of safer and better vaccines that could help in eradicating some of the infectious diseases for which vaccines have not yet been developed.

Allergy represents another example where disease progression can be potentially manipulated by the targeting of antigen to TLRs. Allergy is a Th2-mediated hypersensitivity response in which the antibody response to an allergen is dominated by IgE. The strategy being used to treat allergic responses is to vaccinate in the presence of adjuvants that activate specific TLRs to change the Th1/Th2 balance. This is shown in Fig. 3, in which the experimental animals were exposed to an allergen along with bacterial DNA containing CpG motifs. The ligation of TLR9 by CpG DNA oligodeoxynucleotides decreased the amount of antigen-specific IgE (6). This observation can be explained in part by the increase in the Th1 response caused by TLR9 activation. Therefore, adjuvants that target TLRs have the potential to improve cell-mediated immune responses (Fig. 2) or decrease the incidence of Th2-associated allergic reactions (Fig. 3). Agonists of TLR7/8 have been shown to reduce lung inflammation in mice sensitized with OVA (67). Because of differences in TLR expression between mice and humans, agonists of TLR 7/8 have been used to enhance human immune responses similar to TLR9 in the mouse. Therefore, the strategic combination of TLR agonists and antigen has the potential to change the way we develop vaccines and therapeutics.

Formulation of Next Generation Vaccines

Another challenge in the development of vaccines is delivering antigen and PRR ligands to the lymph node, where immune responses are initiated. New developments in biomaterials are being applied to vaccinology to deliver antigen and adjuvant to the relevant immune cells. An example of this strategy is shown in Fig. 4, where Tacken and colleagues (58) demonstrated that nanoparticles containing TLR ligands along with an antigen could be specifically delivered to DCs by coating the nanoparticles with an antibody to a DC surface receptor called DEC-205. These particles efficiently induced a Th1 response, resulting in high levels of IFN-γ being produced from antigen-specific T cells. New strategies to target antigens and TLR agonists to the appropriate cells (DCs) or tissue (lymph node) hold promise for the improvement of vaccines and CMI responses. A more efficient priming of the immune system at lower antigen doses through targeted antigen delivery has the potential to reduce vaccine toxicity.

Preserving Cytosolic Sanctity Through NOD-Like Receptors

A second general class of PRRs detects microbial/danger signals in the cytosol. NOD-like receptors (NLRs; nucleotide-binding domain, LRR-containing receptors) form signaling platforms such as inflammasomes or NOD signalosomes in response to cytosolic danger signals (36). These proteins are typically composed of a COOH-terminal LRR domain, a nucleotide-binding/oligomerizing domain called NACHT, and an NH2-terminal effector domain that could be a caspase recruitment domain (CARD), pyrin domain (PYD), or baculovirus inhibitor of apoptosis protein repeat (BIR) domain (55). Based on the NH2-terminal effector domains, NLRs can be subcategorized into the NLR family (containing a transactivating domain, CITA), NLRB family (containing the BIR domain; NAIPs), NLRP family (containing a PYD; NALPs), and NLRC family [containing the CARD domain, NODs and ice protease-activating factor (IPAF)]. The effector domains of these receptors initiate innate immune responses in the cytosol. Their main functions include the activation of NF-κB, MAPK, and caspase-1. Although the exact mechanism of activation is not well understood, their activation is thought to occur through the recognition of a PAMP, which allows ATP-mediated oligomerization of NLRs through the NACHT domain. This allows homophilic interactions of NLRs with other PYD/CARD-containing effector molecules/adaptors (35).
The first NLRs to be identified and characterized were NOD1 and NOD2. These receptors recognize the bacterial cell wall components peptidoglycan and muramyl dipeptide, respectively. Binding of these components leads to the oligomerization of NOD1 or NOD2 to recruit receptor-Interacting serine-threonine protein kinase 2 (RIPK2), through a homophilic CARD-CARD interaction. RIPK2 is cross-activated, leading to NF-κB activation (19). The mechanism of the activation of the MAPK pathways is not well understood but is thought to occur through the interaction of CARD9 with NOD1/2 (24). The third important function of NLRs is the formation of inflammasomes, which leads to the activation of caspase-1. NLRs form three prototypical Inflammasomes: NLRP3, NLRC4, and NLRP1 (36). Common to all three are the requirement of the adaptor molecule apoptosis-associated speck-like protein containing a CARD (ASC). ASC binds to the oligomerized NLRs through homophilic FYD interactions and recruits procaspase-1. As procaspase-1 molecules are brought into close contact with one another, they are converted to active caspase-1 through autocatalytic cleavage. An important consequence of caspase-1 activation is the processing and secretion of IL-1β and IL-18. These cytokines are involved in 1) activating innate immunity by activating cyclooxygenase 2, inducible nitric oxide synthase, and phospholipase A and 2) promoting early inflammation through the expression of chemokines and cell surface integrins on endothelial cells. Another consequence bought about by excess caspase-1 activation is a type of inflammatory programmed cell death termed pyroptosis, which can be observed when macrophages are infected with intracellular bacteria (8).

**NLRP6 Is Critical in the Regulation of the Gut Microbiome**

Tens of trillions of microbes (predominantly bacteria) reside in the distal portion of human intestines (10). The gut microbiota has been shown to play important roles in many different processes. They aid in our digestion, influence our food intake (62), “tone” the immune system (17), prevent allergic responses to food antigens, and control the growth of pathogenic strains of bacteria in the gut. Alterations in the gut microbiome have been linked to Crohn’s disease and ulcerative colitis, and new strategies to replace the gut microbiota of *Clostridium*-infected individuals are proving effective (46). A deficiency of NLRP6 or ASC in mouse colonic epithelial cells resulted in altered gut microbiota characterized by an increase in the bacterial phylum *Bacteroidetes* (Prevotellaceae) and the candidate phylum TM7 (10), and this alteration in the microbiome was primarily due to the lack of IL-18 production. IL-18 production at low levels has been shown to be important for maintaining epithelial cell integrity, thereby preventing bacterial translocation (50). NLRP6 inflammasome-deficient mice have also been shown to have spontaneous intestinal hyperplasia and exacerbation of colitis induced by dextran sodium sulfate. This is primarily due to increased chemokine (C-C motif) ligand (CCL3) expression in response to the gut microbiome, promoting increased cell recruitment and spontaneous inflammation. NLRP6 assembly in the colonic epithelial compartment is thought to be driven by the presence of low levels of unidentified danger signals that accompany the loss of tissue integrity, resulting in the local production of IL-18.

**IL-1 Signaling in Skin and the Regulation of Leishmania Major Effector Responses**

An important consequence of inflammasome activation is the secretion of IL-1. IL-1 has been shown to be important for T cell recruitment and activation. Analogous to the gut microbiome, the skin microbiota also tunes the local immune response and influences T cell maturation. Germ-free mice infected with the cutaneous pathogen *Leishmania major* showed a decrease in effector IFN-γ<sup>+</sup> T cells after infection (40). They also showed a decrease in skin pathology, with smaller lesion sizes and reduced edema and necrosis. This effect was mediated by reduced IL-1 signaling in keratinocytes, resulting in diminished Th17 effector responses. These experiments indicate the important role of IL-1 in the maintenance of tissue fitness and the regulation of local immune/inflammatory response to infections.

**NLR-Mediated Cell Death in Controlling Infections**

Inflammasome-dependent caspase-1 activation can lead to a form of programmed cell death called pyroptosis. Pyroptosis, unlike apoptosis, is characterized by cell death associated with inflammation and the release of inflammatory cytokines (13). Programmed cell death plays an important role in limiting the progression of some infectious diseases. NLRC4-deficient or caspase-1-deficient macrophages become resistant to cell death and therefore susceptible to infections by pathogens such as *Salmonella typhymurium* (14), *Pseudomonas aeruginosa* (54), or *Shigella flexneri* (56). The importance of pyroptosis has also been demonstrated for NAIP5-dependent control of *Legionella pneumophila* (70). Macrophages from NAIP5 mutant mice (A/J) exhibited decreased caspase-1 activation followed by defective pyroptosis and microbial control.

Autophagy is a process where cellular components are degraded in specialized compartments called autophagosomes. Autophagy can regulate cellular responses to infection and influence antigen presentation, phagosome maturation, and cell death. Originally thought to have developed in response to cellular stress, antimicrobial autophagy has now been shown to be an evolutionarily conserved defense mechanism shared by plants, flies, and mammals. It is known to play a vital role in NOD2-mediated control of the intracellular bacteria *S. typhimurium* (23). NLRC4-mediated regulation of autophagy has also been shown to protect host cells from cell death induced by *Shigella* infection (56).

**RIG-I-Like Receptors and Type I IFNs**

Nucleic acids are now appreciated to be potent immunostimulatory molecules, and nucleic acid sensing is central to antiviral defenses. Viral nucleic acids can serve as cytosolic PAMPs and are especially important for the detection of RNA viruses. TLR3 was the first RNA sensor identified to activate IFN regulatory factors (IRFs) and NF-κB (2). However, in vivo antiviral responses to lymphocytic choriomeningitis virus, reovirus, and murine cytomegalovirus were found to be similar between TLR3 knockout and wild-type mice (9). This suggested that other sensors of viral RNA existed. RIG-I and melanoma differentiation-associated protein (MDA)-5, cytosolic proteins containing a COOH-terminal DEXD/Hbox-containing RNA-helicase domain and two NH<sub>2</sub>-terminal CARDs,
have been identified as receptors for viral RNA capable of inducing TLR-independent induction of type I IFNs (29, 69). Initially characterized as a double-stranded (s)RNA-binding protein that triggered IFN induction, RIG-I has also been shown to be important in mounting antiviral responses against the positive-strand ssRNA virus Japanese encephalitis virus as well as negative-strand ssRNA viruses such as Newcastle disease virus, vesicular stomatitis virus, Sendai virus, and influenza virus. MDA-5 senses the presence of the positive-strand ssRNA picornavirus encephalomyocarditis virus. RIG-I preferentially recognizes RNA sequences marked with 5′ triphosphorylated (5′-ppp) ends, which serve, in part, to define a nonself RNA PAMP (5). It has been suggested that MDA-5 preferentially recognizes high-molecular-weight poly(I:C) fragments, whereas RIG-I shows a preference for shorter RNA fragments and can also bind to ssRNA (30). Once the RNA ligands are bound to the receptors, RIG-I or MDA-5 recruit CARD adaptor inducing IFN-β (CARDIF) through their CARD domains. CARDIF forms a platform from which the NF-κB pathway is activated through TRAF6 and the IRF pathway through TRAF family member-associated NF-κB activator-binding kinase (TBK)-1. It has been shown that aberrant regulation of RIG-I-like receptor signaling not only leads to defective antiviral immune responses but also an increase in the severity of autoimmune diseases like type 1 diabetes (42), systemic lupus erythematosus (47), and Crohn’s disease (15).

**Cytosolic Sensing of DNA**

The identification of cytosolic DNA sensors is fairly recent, and most of the sensors have not been extensively studied. Although the discovery of TLR9 and its characterization in recognizing bacterial CpG DNA (22) initially explained the immunostimulatory properties of DNA, TLR-independent IFN production after cytosolic delivery of DNA hinted at the presence of other receptors for DNA (25). As of now, six intracellular receptors have been implicated in DNA recognition by the host. These include absent in melanoma (AIM2), RNA polymerase III, DEXD/H box helicases (e.g., DHX9 and DHX36), DNA-dependent activator of IFRs (DAI; also called Z-DNA-binding protein 1), IFN-inducible protein IFI16, and DNA-dependent activator of IRFs (DAI; also called DAI) through their CARD domains. CARDIF forms a platform from which the NF-κB pathway is activated through TRAF6 and the IRF pathway through TRAF family member-associated NF-κB activator-binding kinase (TBK)-1. It has been shown that aberrant regulation of RIG-I-like receptor signaling not only leads to defective antiviral immune responses but also an increase in the severity of autoimmune diseases like type 1 diabetes (42), systemic lupus erythematosus (47), and Crohn’s disease (15).

**Cytosolic Sensing of DNA**

The identification of cytosolic DNA sensors is fairly recent, and most of the sensors have not been extensively studied. Although the discovery of TLR9 and its characterization in recognizing bacterial CpG DNA (22) initially explained the immunostimulatory properties of DNA, TLR-independent IFN production after cytosolic delivery of DNA hinted at the presence of other receptors for DNA (25). As of now, six intracellular receptors have been implicated in DNA recognition by the host. These include absent in melanoma (AIM2), RNA polymerase III, DEXD/H box helicases (e.g., DHX9 and DHX36), DNA-dependent activator of IFRs (DAI; also called Z-DNA-binding protein 1), IFN-inducible protein IFI16, and DNA-dependent activator of IRFs (DAI; also called DAI) through their CARD domains. CARDIF forms a platform from which the NF-κB pathway is activated through TRAF6 and the IRF pathway through TRAF family member-associated NF-κB activator-binding kinase (TBK)-1. It has been shown that aberrant regulation of RIG-I-like receptor signaling not only leads to defective antiviral immune responses but also an increase in the severity of autoimmune diseases like type 1 diabetes (42), systemic lupus erythematosus (47), and Crohn’s disease (15).

**Cytosolic Sensing of DNA**

The identification of cytosolic DNA sensors is fairly recent, and most of the sensors have not been extensively studied. Although the discovery of TLR9 and its characterization in recognizing bacterial CpG DNA (22) initially explained the immunostimulatory properties of DNA, TLR-independent IFN production after cytosolic delivery of DNA hinted at the presence of other receptors for DNA (25). As of now, six intracellular receptors have been implicated in DNA recognition by the host. These include absent in melanoma (AIM2), RNA polymerase III, DEXD/H box helicases (e.g., DHX9 and DHX36), DNA-dependent activator of IFRs (DAI; also called Z-DNA-binding protein 1), IFN-inducible protein IFI16, and DNA-dependent activator of IRFs (DAI; also called DAI) through their CARD domains. CARDIF forms a platform from which the NF-κB pathway is activated through TRAF6 and the IRF pathway through TRAF family member-associated NF-κB activator-binding kinase (TBK)-1. It has been shown that aberrant regulation of RIG-I-like receptor signaling not only leads to defective antiviral immune responses but also an increase in the severity of autoimmune diseases like type 1 diabetes (42), systemic lupus erythematosus (47), and Crohn’s disease (15).

**Intracellular PRRs and the Generation of CMI Responses: Listeria Monocytogenes**

Listeria monocytogenes is an intracellular bacterium that has been widely used as a model organism to study the robust induction of CMI. This activation can be explained by the activation of a number of PRRs by L. monocytogenes. Infection of mammalian host cells with L. monocytogenes is known to activate at least three innate immune pathways: 1) MyD88-dependent TLR signals emanating from the phagosome, resulting in the activation of NF-κB; 2) activation of the STING/IRF3 pathway, leading to the production of type I IFNs; and 3) AIM2-dependent activation of caspase-1, leading to the maturation and secretion of IL-1β, IL-18, and pyroptosis (65). The complexity of the innate immune responses to Listeria suggests that no single innate pathway can adequately initiate and maintain optimal adaptive immune responses to L. monocytogenes.

During a Listeria infection, bacteria are quickly taken up into the phagosomes of macrophages. One of the first PRRs activated is TLR2, which recognizes bacterial lipoproteins (61). Flagellin isolated from Listeria has also been shown to activate TLR5 (21). In a MyD88-dependent manner, these receptors activate the transcription of inflammatory cytokines such as IL-12, TNF, and IL-6. The importance of these signals is emphasized in MyD88 knockout mice, which are more susceptible to Listeria infections compared with wild-type mice. After uptake, Listeria escape the phagosome and enter the cytosol of host cells. Cyclic nucleotides have been demonstrated to be important in regulating the expression of virulence genes and many aspects of bacterial physiology. Secretion of cyclic di-AMP from the bacteria has recently been shown to activate STING-dependent transcription of type I IFNs (66). Mice with a mutated STING receptor have been shown to induce a defective IFN-β response. IFN-β responses are important in promoting resistance to Listeria infections by sensitizing host cells to apoptosis. The inefficient bacteriolysis results in the release of low levels of bacterial DNA into the cytosol of host cells, which triggers the activation of caspase-1 through the assembly of the AIM2 inflammasome (48). This initiates pyroptosis of host cells. A strain of Listeria engineered to secrete a fusion protein that activates NLRC4/IPAF is highly
attenuated in wild-type mice (64) but is pathogenic in caspase-1-deficient mice, indicating the importance of inflammasome-dependent mechanisms in controlling intracellular bacterial pathogenesis.

The ability of Listeria to stimulate multiple innate immune pathways has provided clues as to how to develop vaccines to induce CMI. One can hypothesize that it is the stimulation of multiple PRRs that makes L. monocytogenes an effective inducer of CMI and therefore a promising candidate for use as a live bacterial vaccine vector for the treatment of chronic or intracellular infections. Thus, our understanding of how Listeria activates host PRRs may inform future vaccine strategies.

Chemotaxis and Cell Migration

An effective host response to foreign pathogens requires the induction of inflammation by the innate immune system. Inflammation is induced through the coordinated recruitment of immune cells to the site of infection. Leukocytes egress out of the circulatory system to the site of inflammation through a process called extravasation. This process involves the rolling of leukocytes on the vessel endothelium followed by firm adhesion and transendothelial migration through fenestrations in the capillary wall. Two sets of receptors mediate this process: 1) selectins are responsible for low-affinity adhesions that mediate leukocyte rolling and 2) integrins are responsible for tight adhesion to the endothelium. Once leukocytes have exited the blood, the control of cellular recruitment is mediated by chemoattractants, which fall under three general classes: 1) formylated peptides, 2) complement anaphylatoxins, and 3) chemokines. All of these chemoattractants induce cellular recruitment by binding to G protein-coupled seven-transmembrane protein receptors on immune cells. Chemokines transduce signals through Gq and Goq, which signal for cAMP production, and phospholipase C activation, resulting in Ca2+ fluxes in the cell. Chemokines promote cellular motility by inducing cytoskeleton reorganization and actin polymerization.

In bacteria during the process of translation, the first amino acid to be produced is formylated methionine. The degradation of bacteria results in the release of short peptides with formylated methionine. Neutrophils are highly motile leukocytes that respond to formylated peptides. Neutrophils destroy bacteria through the production of ROS and antimicrobial peptides. In addition to attracting neutrophils to the sites of infection, formylated peptides also induce the activation of neutrophils to produce increased ROS production as well as the release of lysosomal vesicles into the extracellular matrix through a process that involves phospholipase C activation and Ca2+ signaling (33). Complement anaphylatoxins are also important contributors to leukocyte migration. The activation of complement results in the proteolytic cleavage of C3 and C5, releasing C3a and C5a. These peptides bind to C3a and C5a receptors on leukocytes and induce chemotaxis. C5a binding to its receptor can also induce histamine release, an increase in ROS production, and enhanced vascular permeability (63). Thus, these chemotactic molecules can make important direct contributions to host defense, and they also organize immune cells to promote adaptive immune responses in response to vaccines or infections.

Chemokines

There are >40 chemokines, which can be divided into 4 general groups, based on the number and spacing of the first two conserved cysteine residues in the NH2-terminus: the CXC, CC, C, and CX3C families (where X is any amino acid) (12). Chemokines can be functionally divided into inflammatory chemokines (e.g., CCL2, CCL5, chemokine (C-X-C motif) ligand (CXCL)8, chemokine (C-X3-C motif) ligand 1, etc.) and homeostatic chemokines (CXCL12, CCL1, CCL25, etc.). Homeostatic chemokines play important roles in the recruitment of innate immune cells like monocytes, neutrophils, DCs, natural killer cells, etc. They are usually known to bind to more than one chemokine receptor, which sometimes indicates redundancy in their function. Homeostatic chemokines, apart from regulating cell development, also influence the migration of APCs and effector T cells to and from lymph nodes. Mice deficient in homeostatic chemokines display abnormal lymph node architecture.

Differential expression of chemokine receptors on immune cells can serve to control their recruitment to sites of inflammation. For example, memory T cells express CCR7 to allow their homing to the lymph node. Chemokine receptor expression can also be used as biomarkers specific to individual cells or subpopulations of cells. This has helped immunologists to identify and study cell types that appear to be morphologically similar but vary greatly in function (53). Human monocytes can be divided into several subpopulations that are functionally distinct (16). Of the two major monocyte subpopulations (53), the so-called “classical” monocytes express high levels of CD14 and lack CD16. In healthy humans, these cells can account for 80–90% of the monocytes in blood. The “nonclassical” monocytes express lower levels of CD14 but high levels of CD16. These cells comprise 10–15% of monocytes in blood. There are subtle differences in these cells with regard to size and granularity, but their expression of chemokine receptors is dramatically different. The nonclassical population expresses high levels of the chemokine receptor chemokine (C-X3-C motif) receptor 1 (CX3CR1) but low levels of chemokine (C-C motif) receptor 2 (CCR2). The classical population expresses high levels of CCR2 but low levels of CX3CR1. Therefore, the two populations can be easily discriminated by the relative expression of these two chemokine receptors. Chemokine receptor expression can also provide clues as to the functional activity of a given cell type. The classical monocytes expressing CCR2 are thought to be important in inflammatory responses because the chemokine CCL2 is rapidly released at sites of inflammation (18).

Innate Immunity, Immunopathology, and the Maintenance of Homeostasis

In addition to their well-described role in pathogen sensing and host defense, innate immune cells play a major role in maintaining tissue homeostasis. These cells maintain a balance between immune stimulation and immunoregulation. Macrophages provide an ideal example of this. They are frequently exposed to activating stimuli, in the form of necrotic tissue debris, environmental stimuli in the lungs, on the skin, and in the gut, and they are effector cells that respond to adaptive immune responses. To maintain homeostasis, these activating stimuli must be counterbalanced by inhibitory signals. Some of
these regulatory signals include the uptake of apoptotic cells (11) and exposure to immunomodulatory compounds, such as prostaglandins (52), adenosine (20), or glucocorticoids. These signals inhibit activating responses, and, without them, the activation of innate immune responses would continue unabated, leading to immunopathology. The plasticity of macrophages allows them to change their physiology in response to these diverse signals and regulate immune homeostasis. They can be activated to kill intracellular pathogens, or, alternatively, they can resolve inflammatory processes and promote wound healing (39). The duration of their activation is dependent on the strength and persistence of the activating stimuli. Improper functioning of the regulatory pathways that limit activation has been implicated in diseases such as obesity (4), type 2 diabetes (43), and rheumatoid arthritis (57).

In summary, the innate immune system has a diverse set of cell types expressing many PRRs, both surface expressed and intracellular. As our understanding of innate immunity and PRRs increases, so does our ability to exploit innate immunity to produce better vaccines or find new ways to manipulate adaptive immune responses. This can lead to a decrease in infectious diseases or the prevention of autoimmunity.

DISCLOSURES

D. M. Mosser is the founder and CEO of a startup biotechnology company that works in the field of inflammation.

AUTHOR CONTRIBUTIONS

Author contributions: R.S. drafted manuscript; D.M.M. edited and revised manuscript; D.M.M. approved final version of manuscript.

REFERENCES

4. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 17: 4–12, 2006.


