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Intracellular sensors of microbes and danger

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The editors of this volume of Immunological Reviews respectfully acknowledge the passing of Jürg Tschopp, who died suddenly on March 22, 2011. A towering figure in the field, Jürg made many important contributions to our understanding of cell death and inflammation signaling pathways. His seminal description of the inflammasome has not only had major impact on scientists working in innate immunity but also influenced many interested in related fields. The impact of Jürg's work was immense, ranging from improving our understanding of basic immunologic principles to providing novel insights leading to new modes of therapy for important human diseases. Jürg was not only a colleague but also a friend of several contributors to this volume. He will be missed.

The inflammatory response is critical for host defense against invasive pathogens. Upon infection, a cascade of signals leads to recruitment of acute inflammatory cells, particularly neutrophils and macrophages. Similar to the elimination of infectious agents, inflammation is also vital for tissue repair resulting from cellular injury or deposition of particulate matter that occurs in the absence of infectious agents. Similar to microbial induced inflammation, sterile inflammation is marked by the recruitment of neutrophils and macrophages and the production of pro-inflammatory cytokines and chemokines that mediate the removal of cellular debris or foreign matter and ultimately tissue repair.

Eukaryotic hosts deploy an arsenal of defense mechanisms to counter invading microbes. Upon infection, initial recognition of pathogenic organisms and induction of antimicrobial responses are mediated by germline-encoded pattern recognition receptors (PRRs). These PRRs recognize conserved moieties found in microbial organisms, which are often referred to as pathogen-associated molecular patterns (PAMPs). Although these structural motifs are involved in the recognition of pathogens, they are also expressed by non-pathogenic microbes, and therefore the presence of PAMPs cannot explain the discrimination between pathogenic and non-pathogenic microorganisms by the host immune system. Certain PRRs also sense host-derived non-microbial molecules that are released during

cellular injury or activities induced by cellular damage. These endogenous molecules have been termed damage-associated molecular patterns (DAMPs). As the inflammatory response induced in response to DAMPs is similar to that observed during microbial infection, it was expected that certain PRRs are also involved in the induction of sterile inflammation. At least five major classes of PRRs have been identified to date. They include transmembrane Toll-like receptors (TLRs), which are located at the extracellular surface or within endosomes and C-type lectin receptors, which are also membrane bound and characterized by the presence of a carbohydrate-binding domain (Fig. 1). In addition, there are three families of cytoplasmic PRRs: Nod-like-receptors (NLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and Aim2 (Fig. 1). NLRs are defined by a tri-partite structure, namely (i) an N-terminal caspase recruitment domain (CARD), a pyrin domain (PYD), acidic transactivating domain, or baculovirus inhibitor repeat (BIR) that mediate downstream protein-protein interactions, (ii) a centrally located nucleotide-binding oligomerization (NOD) domain that mediates self-oligomerization important during activation, and (iii) C-terminal leucine-rich repeats (LRRs) that dictate ligand specificity. Several NLRs participate in the formation and activation of inflammasomes, multi-protein platforms that direct the activation of caspase-1, a protease that promotes the secretion of the pro-inflammatory cytokines IL-1β and IL-18. RLRs are primarily involved in antiviral responses, and Aim2 belongs to a family of receptors characterized by the presence of a PYD and DNA-binding HIN module involved in the detection of cytosolic microbial DNA. Following activation by microbial or endogenous molecules, these innate sensors activate downstream signaling pathways, including nuclear factor-κB, mitogen-activated protein

kinase, type I interferon pathways, and the inflammasomes, which result in the production of pro-inflammatory cytokines and chemokines important in the inflammatory and antimicrobial response (Fig. 1). This volume of *Immunological Reviews* is focused on emerging knowledge about several aspects of intracellular PRRs including mechanisms of activation, signaling pathways, function in sterile and microbial inflammation, and role in disease.

The presence of PRRs located in the extracellular surface and in the cytoplasm raises questions about the reason for the development of sensors with different cellular localization in both animals and plants. It has been assumed that the location of the PRRs could dictate the type of microbe the innate immune system can sense. For example, cell surface receptors such as TLRs could be primarily activated by extracellular bacteria, whereas cytoplasmic PRRs could be involved in the recognition of microbes with an intracellular lifestyle such as bacteria capable of breaching surface or endosomal membranes or viruses that replicate intracellularly. In their article, Clarke and Weiser (1) point out that it is becoming clear that many extracellular bacteria can be sensed by cytoplasmic PRRs. For example, Clarke and Weiser (1) review evidence that the extracellular bacteria *Staphylococcus aureus* and *Streptococcus pneumonia* produce pore-forming toxins that facilitate delivery of peptidoglycan fragments into the host cytosol which promote Nod1 or Nod2 activation. It is more likely therefore that the presence of extracellular and cytoplasmic PRRs serves another purpose, such as to promote discrimination between non-pathogenic and pathogenic microorganisms by the innate immune system. In support of this notion, Fontana and Vance (2) focused their article on the intracellular pathogen *Legionella pneumophila* to provide evidence for a two-signal model of activation of the innate immune system that could serve to selectively detect infectious microbes. There is evidence that toxins produced by pathogenic bacteria promote bacterial growth in the host, but at the same time they contribute to pathogen recognition by cytoplasmic PRRs. For example, pore-forming or membrane-damaging toxins including those produced by *S. aureus* and *Vibrio cholera* induce the activation of Nlrp3, another host cytoplasmic sensor that directs the activation of caspase-1, leading to interleukin-1β (IL-1β) and IL-18 secretion (3, 4). Similarly, Nlrp1b, another NLR family member that activates caspase-1, is activated by lethal toxin, a bipartite toxin secreted by *Bacillus anthracis* (5). Several Gram-negative bacteria, including *L. pneumophila*, *Pseudomonas aeruginosa*, and the enteric pathogens *Salmonella typhimurium* and *Shigella flexneri*, use type III or IV secretion systems to form pores in host cell membranes that mediate translocation of a wide array of virulence factors (effectors proteins) into the cell cytosol, which is critical for pathogen colonization (6). Importantly, these bacterial secretion systems promote the delivery of small amounts of flagellin into the host cytosol where they induce the activation
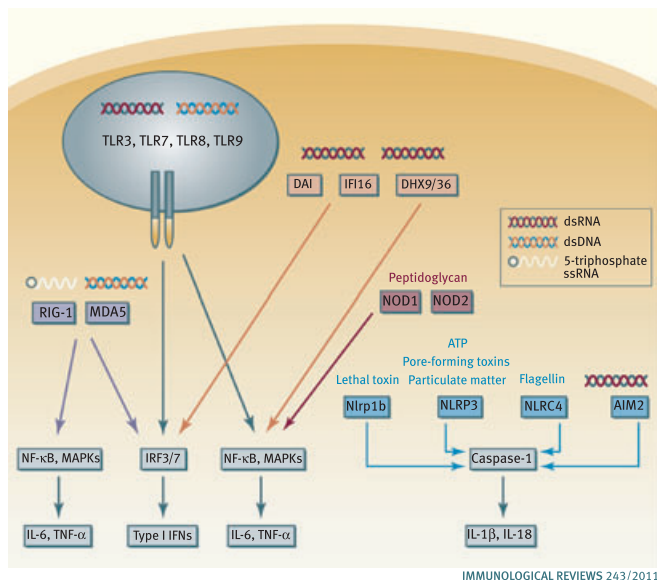

Fig. 1. Intracellular sensors for microbes and danger and their signaling pathways. More details about the sensors and their signaling pathways are provided in the articles of this volume.

of Nlrc4, another NLR family member involved in caspase-1 activation (7, 8). Collectively, these observations strongly suggest that microbial recognition via cytoplasmic PRRs serves to discriminate between non-pathogenic and pathogenic microbes and to tailor the host response accordingly.

Pioneering studies provided evidence in support of TLR-independent cytosolic sensing of bacterial molecules by epithelial and macrophages (9, 10). These initial studies were confirmed when the first cytoplasmic PRR in animal cells, Nod1, was identified in 1999 and shown later to be activated by bacterial products (11). Specifically, Nod1 and Nod2 were found to sense conserved molecules derived from the synthesis, recycling, or degradation of bacterial peptidoglycan (12, 13). It is now clear that peptidoglycan, a key component of the cell wall in both gram-positive and gram-negative bacteria, is important not only for maintaining the structural integrity of the bacterial cell but also for recognition of bacteria by the innate immune system. Sorbara and Philpott (14) review and discuss how the innate immune system is activated by conserved motifs present in peptidoglycan. Furthermore, they describe how peptidoglycan recognition by intracellular PRRs is critical for host defense and dysregulation of these signaling pathways increases susceptibility to inflammatory disease. Nucleic acids are also key molecules that are used by host cells to sense microbes and to activate the innate immune system. Several classes of PRRs with different intracellular location that recognize microbial and endogenous nucleic acids have been described. Kawasaki *et al.* (15) provide a comprehensive description and discussion of intracellular PRR signaling pathways involved in nucleic acid recognition as well as their role in the development of autoimmunity. In a similar vein, Wang *et al.* (16) focus on the mechanisms by which viral dsRNA is detected by intracellular and cytoplasmic sensors of the innate immune system and elicit production of type I interferons. These authors discuss why these dsRNA sensors are differentially expressed in distinct host cells, are located in different intracellular compartments, and play non-redundant roles in controlling viral infection. A major class of intracellular PRRs involved in viral recognition is RLRs. Kato and colleagues (17) review our current knowledge about molecular and structural aspects of RLRs including their ligands, mechanisms of activation, and regulation of signaling pathways. While RLRs sense primarily viral RNA molecules, several intracellular PRR pathways have been identified that recognize microbial and endogenous DNA. Barber (18) discusses the innate sensors of DNA and their signaling pathways. Fitzgerald (19) discusses the role of the Aim2 inflammasome in host defense against viruses and bacteria via cytosolic recognition of microbial DNA. In addition, this author reviews emerging knowledge about additional members of the HIN-200 protein family in the activation of type I interferon responses.

The inflammasome is a multi-protein platform that is characterized by its ability to activate caspase-1, which in turn proteolytically cleaves pro-IL-1β and pro-IL-18 into their mature biologically active forms. Inflammasomes are named by the PRR that regulates its activity and dictates the nature of the upstream activating stimulus. To date, there are four known *bona fide* inflammasomes, based on their dependence on caspase-1 activation for IL-1β and IL-18 production: the Nlrp1, Nlrp3, Nlrc4, and Aim2 inflammasomes. Nlrp1, Nlrp3, Nlrc4, and Aim2 are PRRs that can sense PAMPs and also DAMPs in the case of Nlrp3. With the exception of Aim2, which is a member of the interferon-inducible HIN-200 protein family, the other known inflammasomes all contain a PRR that belong to the NLR family. Horvath and colleagues (20) provide a comprehensive review about the different intracellular PRRs that have been linked to inflammasome formation, their putative microbial and endogenous agonists and their mechanism of activation, whereas Gross *et al.* (21) focus on the Nlrp3 inflammasome. The latter authors discuss different models of Nlrp3 activation including the role of K$^+$ efflux, reactive oxygen species, and lysosomal destabilization. Finally, they discuss different cellular mechanisms that have been suggested to regulate the activity of the Nlrp3 inflammasome and caspase-1 targets other than IL-1β and IL-18. The articles by Lemmans *et al.* (22) and Lamkanfi *et al.* (23) also are devoted to review our understanding of the Nlrp3 inflammasome, but they focus on the role of the inflammasome in the development of disease including that associated with ischemic-reperfusion injury, atherosclerosis, inflammatory bowel disease, Alzheimer's disease, and metabolic disorders such as diabetes and gout. In contrast, the article by Broz and Monack (24) discusses the role of the inflammasomes in host defense with a focus on those activated by *S. typhimurium* and *Franciscella tularensis*. These authors also review how microbes can manipulate inflammasome activity and discuss their evidence for the existence of two functionally distinct caspase-1 complexes.

Cell death is often associated with host responses to microbial pathogens. Classically, cell death was divided into two major classes, necrosis and apoptosis, that exhibit distinct cellular and molecular features. Apoptosis represents an orchestrated caspase-signaling cascade that ultimately leads to formation of apoptotic bodies that can be cleared effectively by phagocytes. In contrast, necrosis involves cellular and organelle swelling and, most importantly, rupture of the plasma membrane, resulting in the release of intracellular molecules that can elicit an inflammatory response. Rock and colleagues (25) discuss the endogenous molecules or DAMPs that elicit inflammation as well as the innate sensors and adaptive immune responses that are activated in response to dying cells. Several intracellular bacteria induce pyroptosis, a specialized form of macrophage cell death with features of both

necrosis and apoptosis. Upon bacterial infection, pyroptosis is triggered via the activation of caspase-1, which induces the release of IL-1β and IL-18 as well as intracellular inflammatory contents to stimulate additional inflammatory signaling pathways. Miao *et al.* (26) review our current understanding of the mechanisms by which intracellular bacteria activate pyroptosis and the intracellular PRR signaling pathways involved. In addition, these authors discuss the role of pyroptosis in host defense with a focus in the *S. typhimurium* model of infection. There is mounting evidence of evolutionary conservation between innate immunity and cell death signaling pathways. For example, NLRs were identified based on structural homology to Apaf-1, the activator of caspase-9 in the mitochondrial apoptosis pathway (27). Tal and Iwasaki (28) discuss the emerging role of the mitochondria in the activation of innate signaling pathways and specifically those involved in the antiviral responses. Saleh (29) reviews the evolutionary connection between apoptotic cell death and innate immunity and recent data-linking NLR signaling and the cell death machinery. Because the symbiotic theory predicts that the mitochondrion is an organelle derived from gram-negative bacteria, it is possible that ancestral mechanisms associated with bacteria are involved in the development of the cellular machineries for cell death and defense against microbial pathogens in multicellular organisms.

The immune system employs an inflammatory response to protect the host from invasive pathogens as well as for initiating tissue repair that occurs in the absence of infectious triggers. The 17 reviews within this volume of *Immunological Reviews* collectively cover our current understanding of intracellular sensors of microbes and danger, including the mechanisms of PRR activation, the signaling pathways utilized, the role of PRRs in sterile and microbial inflammation, and their role in disease. While these sensors are critical for host defense, their inappropriate activation has also been linked to inflammatory disorders. Hence, a more complete appreciation of these receptors and their signaling pathways will provide important insights into new therapeutic modalities that can either enhance function, thus potentiating responses to pathogens or inhibit function, thereby lessening the deleterious effects of unchecked inflammatory responses.

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