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Inflammasomes in Host Defense and Autoimmunity

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Abstract: Inflammasomes are large multi-protein complexes that control host defense and inflammation during infections with pathogens. Their clinical importance however reaches beyond infectious disease, since dysregulated inflammasome activity has also been linked to many auto-inflammatory disorders. This article gives a short overview on the basics of inflammasome signaling, their activation mechanisms and their role in autoimmunity.

Keywords: Autoimmunity · Infectious diseases · Inflammasomes · Inflammation · Innate immunity

The innate immune system provides the first line of defense against invading pathogens by initiating the onset of the inflammatory response, which promotes pathogen removal and restores tissue homeostasis. A critical step is to first detect the presence of a pathogen in the host organism. This is achieved by so-called pattern-recognition receptors (PRRs), which monitor the extra- and intracellular compartments of host cells for the presence of pathogens. PRRs recognize conserved microbial molecules known as pathogen-associated molecular patterns (PAMPs) that are generally only made by microbes and not host cells thus allowing a distinction between self and non-self.[1] At the same time, PRRs also detect the presence of mislocalized self-molecules, like DNA or certain cytosolic proteins, which act as indicators of cellular damage or stress and are thus referred to as danger-associated molecular patterns (DAMPs).

The first group of PRRs that was characterized were the Toll-like receptors (TLRs), transmembrane proteins that detect PAMPs and DAMPs in the extracellular compartment or within endosomes.[2] Ligand recognition by TLRs results in the initiation of conserved signaling pathways leading to the production of inflammatory cytokines and interferons; signaling molecules that induce inflammation and coordinate anti-microbial host defense reactions. Many successful pathogens, among them viruses and bacteria, however do not reside extracellularly but invade and replicate within host cells. Thus the host cell cytosol also features a diverse set of PRRs. Notable among these are receptors that do not induce a transcriptional response, but initiate the assembly of large signaling complexes commonly referred to as inflammasomes (Fig. 1).

Inflammasomes are assembled by members of the NOD-like receptors (NLR), PYD-, and HIN- domain containing (PYHIN) proteins family and TRIM-family members. These proteins are characterized by distinct functional domains that participate in ligand sensing, oligomerization, and the recruitment of downstream signaling components. Assembly of an inflammasome involves ligand-induced activation of the receptor protein, followed by self-oligomerization and the exposure of signaling domains, which can be either a PYD (Pyrin Domain) or a CARD (Caspase Recruitment Domain).[3] This results in the recruitment of a small signaling adaptor protein called ASC, which consists only of a PYD and CARD. ASC is unique in its function, in that upon recruitment to activated receptors, it starts to oligomerize into long helical filaments *via* its PYD, which expose its CARD at their surface.[4] This step acts as a signal amplification mechanism since it allows a single receptor oligomer to initiate the generation of large number of freely exposed CARDs in a short time.[5] These free CARDs are important for the recruitment of pro-caspase-1, the inactive form of the inflammasome effector protease caspase-1. Dimerization and auto-proteolysis results in the generation of a catalytically active caspase-1 heterotetramer, which is released from the complex and mediates downstream signaling.

Active caspase-1 is an effective protease and has been shown to cleave a number of target proteins to exert its biological function. Over the years two main outcomes have been characterized in detail, which are the proteolytic maturation and release of certain cytokines and the induction of a lytic inflammatory cell death, called pyroptosis.[6] Proteolytic maturation of cytokines is a well-recognized function of caspase-1 and indeed the protease was initially identified as ICE (Interleukin-converting enzyme),[7] since it was responsible for cleaving the biologically inactive pro-form of interleukin (IL)-1β (pro-IL-1β) into its mature, secreted form. IL-1β is a major driver of inflammation in the body, and responsible for activating immune cells and recruiting them to the site of infection. The second function of caspase-1, which is the induction of cell death was identified later, but has also been shown to play an important role in host defense.[8] Pyroptosis effectively kills the infected cells, thus preventing further intracellular replication of pathogens while at the same time exposing them to extracellular immune mechanism such as the complement system and antibodies. The mechanism by which caspase-1 induces pyroptosis has been recently identified; cleavage

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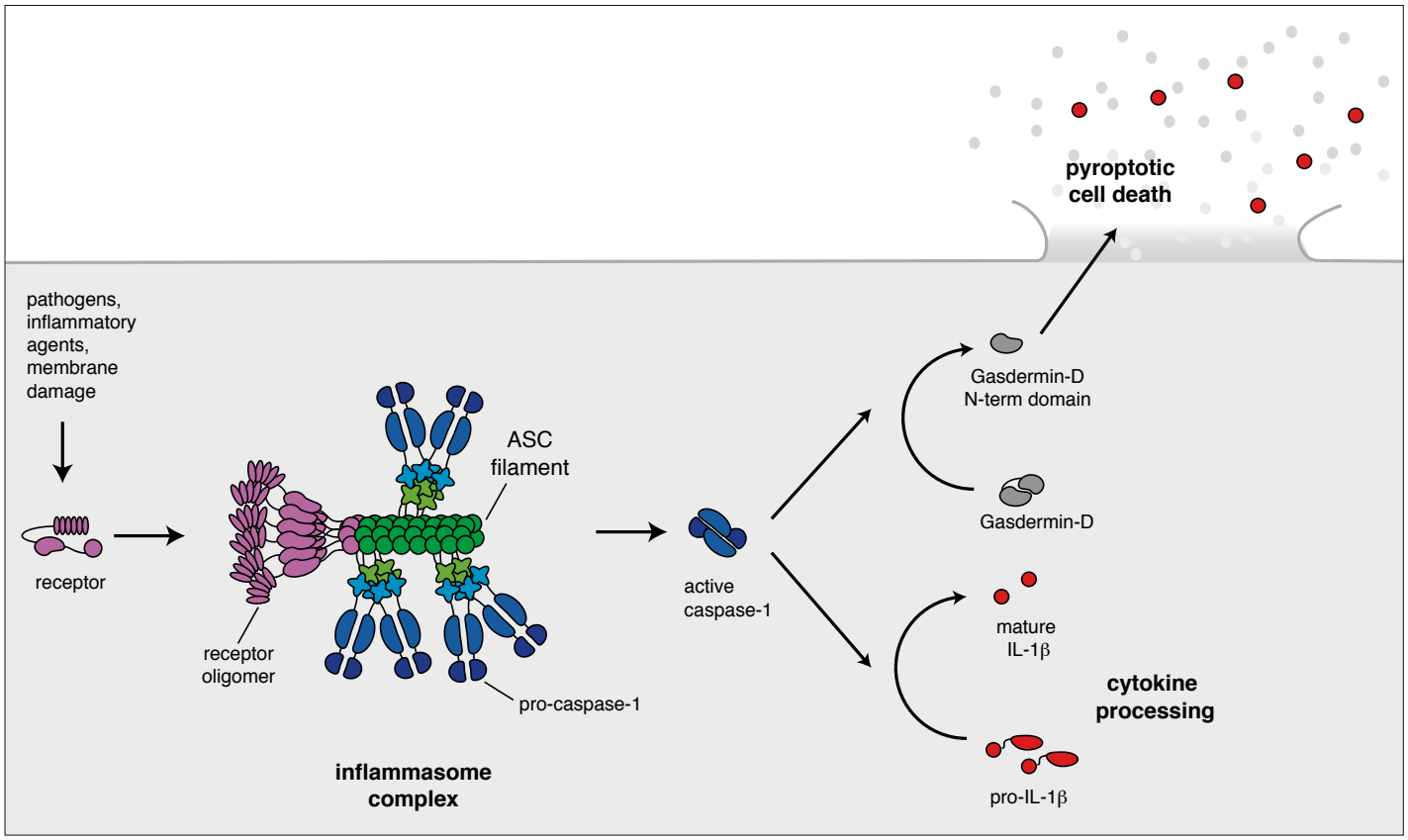

Fig. 1. Schematic representation of inflammasome assembly and signaling. Detection of pathogens or inflammatory stimuli by cytosolic PRRs (*e.g.* NLRP1B, NLRP3, NLRC4, AIM2 or pyrin) activates the receptor (purple) and results in its oligomerization. These receptor oligomers recruit the adaptor ASC (green), which starts to form filaments through its pyrin domain that expose the second domain of ASC, a caspase recruitment domain, on their surface. This results in the recruitment of pro-caspase-1 (blue), which gets activated within the complex. Active caspase-1 is released from the complex and starts to process target proteins. Processing of pro-IL-1β (red) into its mature form by caspase-1 is required for the cytokine to become biologically active. Caspase-1 will in addition induce the processing of Gasdermin-D (gray). Cleavage of Gasdermin-D results in the formation of a cytotoxic N-terminal fragment of Gasdermin-D, which induces pyroptosis, a lytic inflammatory cell death, that is required for the restriction of pathogen replication and the release of processed IL-1β.

of a single protein substrate known as Gasdermin-D is sufficient to induce pyroptosis.[9] This processing event results in the generation of an N-terminal part of Gasdermin-D, which turns out to be highly cytotoxic. How exactly Gasdermin-D kills the cell is still being investigated, but we and other have shown that the N-terminal part of Gasdermin-D can form plasma membrane pores, which presumably result in a rapid loss of the electrochemical gradient and osmotic lysis of the cell.

The main function of inflammasome is to detect pathogens, and this is highlighted by the vast array of microbial ligands that are detected by inflammasome-forming receptors.[10] The best studied among these is NLRC4, which detects bacterial flagellin and several structural components of bacterial type 3 secretion systems (T3SS) through partner proteins known as NAIPs, which directly bind these ligands. Direct ligand binding has also been demonstrated for the receptor AIM2, which features a DNA-binding HIN domain and assembles an inflammasome when detecting DNA in the cytosol. Cytosolic DNA can be an indicator of a viral or bacterial infection, or of damage to the host cell and the leakage of nuclear DNA. Pathogens can however also be detected indirectly, by detecting the damage caused to host cells or the effects of microbial effector proteins. An ingenious mechanism is employed by NLRP1B, which acts as a pseudo-substrate for Lethal Factor (LF) of *B. anthracis*, a protease that normally shuts down innate immune signaling. LF-mediated cleavage of NLRP1B however activates the propensity of the protein to assemble inflammasomes. Pyrin, another of these receptors, can sense the microbe-induced modification of host Rho GTPases, but the mechanism is still unknown, as is the mechanism of NLRP3 activation, a sensor that recognizes pathogens as well as a number of sterile inflammatory stimuli.

Consistent with their specificity for microbial ligands, inflammasomes have been shown in countless animal studies to provide essential protection for the host against viral, bacterial, fungal and even protozoan pathogens. Given the potency of this system, it is hardly surprising that inflammasome assembly needs to be highly regulated,[3] and consistently it has been found that unscheduled and dysregulated inflammasome activation is detrimental to host health. This is evident in a group of auto-inflammatory disorders known as 'inflammasomopathies' caused by activating mutations in inflammasome-forming receptors.[11] Patients suffering from these disorders, including Cryopyrin-Associated Periodic Syndromes (CAPS), Familial Mediterranean Fever (FMF) and macrophage activation syndrome (MAS) experience common symptoms such as fever, rash and sterile inflammation, which are instigated by spontaneous macrophage activation and release of the pyrogenic cytokines IL-1β and IL-18. Furthermore, uncontrolled inflammasome activation has been linked to a number of acquired autoinflammatory diseases, such as gout, pseudogout, asbestosis, silica-mediated pulmonary disorder, atherosclerosis and type II diabetes mellitus. Common to these disorders is that sterile inflammatory triggers, such as uric acid crystals or cholesterol crystals, initiate inflammasome assembly and excess release of IL-1β and inflammation.

Neutralization of the inflammasome thus offers considerable therapeutic promise to reduce detrimental inflammation in inflammasome-related disorders and

inherited autoinflammatory disorders. Anti-IL-1β therapies such as Anakinra (IL-1-receptor antagonist) have been successfully used in the treatment for gout for example, but such therapies are often very costly. Recent studies however have highlighted the potential of small-molecule inhibitors of NLRP3 in regulating inflammasome signaling in mouse models of CAPS and experimental autoimmune encephalomyelitis (EAE).[12] Thus understanding the molecular mechanism of how inflammasomes are assembled and signaled could be instrumental in the identification of new therapeutic targets and could foster the development of new anti-inflammatory therapies for inflammasome-associated diseases based on a range of selective inhibitors of individual inflammasomes.

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