Inflammasomes: far beyond inflammation

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Nearly a decade ago, the concept of inflammasomes was introduced. Since then, the biochemical characterization of the inflammasomes has led to a richer understanding of innate immune responses in the context of infection and sterile inflammation. This has provided the rationale for successful clinical therapies for a spectrum of hereditary periodic fever syndromes and potentially for some metabolic pathologies.

In mammals, multiple recognition systems have coevolved to preserve normal interactions with the commensal flora and to initiate immune responses to invading pathogens and perturbations in tissue homeostasis. Endosomal and extracellular Toll-like receptors recognize mainly pathogen-associated molecular patterns found in microbes. Meanwhile, a multitude of cytosolic receptors recognize not only intracellular pathogen-associated molecular patterns but also the host-derived signals known as 'damage-associated molecular patterns'. The cooperation between these compartmentalized surveillance systems allows organisms to sense and respond to a large number of infectious and sterile insults to the host.

Nod-like receptors (NLRs) are cytosolic pattern-recognition receptors that were initially proposed to regulate inflammation through apoptosis, on the basis of their structural homology to proteins involved in defense against infection in plants (NB-LRR proteins) and to the apoptosis-activating factor APAF-1. However, a decade ago, that initial concept was modified with the discovery of a large molecular platform composed of the NLR protein NLRP1, the adaptor ASC and the proinflammatory caspases 1 and 5 (ref. 1). This large complex was called the 'inflammasome', as it was demonstrated to be required for activation of the proinflammatory cytokine interleukin 1β (IL-1β). Subsequently, many sensor proteins of the NLR family and PYHIN family (pyrin- and HIN-200 domain–containing proteins) have been shown to form inflammasomes in response to a wide variety of damage-associated and pathogen-associated molecular patterns[2] (**Fig. 1**). Today, inflammasomes are recognized as one of the cornerstones of the intracellular surveillance system.

Although the first decade of research in the inflammasome field shed light on the biochemical principles of the assembly, specificity and function of inflammasomes, exciting new evidence has placed inflammasomes at the center stage of complex diseases (metabolic syndrome and carcinogenesis) and physiological processes (regulation of intestinal microbial ecology). This issue of *Nature Immunology* presents four up-to-date reviews of key areas in inflammasome biology[3–6] and lays out the critical open questions now faced in the field.

Regulation of microbial infection

It has become evident that the sensing of pathogens by inflammasomes constitutes a major intracellular defense mechanism against a wide variety of microorganisms ranging from bacteria, viruses and fungi to parasites[7]. However, the activating signals of some of the inflammasomes remain poorly understood. The review by Núñez and colleagues in this issue provides a detailed description of the various inflammasomes and their respective microbial activators; moreover, it discusses emerging concepts in this area of inflammasome biology[3].

The NLRs that participate in the formation of inflammasomes were originally proposed to serve as direct sensors of the cognate activating signals (that is, microbial ligands), but that concept has changed considerably throughout the past few years[2]. The alternative concepts that have been proposed suggest that NLRs may serve as switches and assembly docks for cellular receptors or signals that recognize cellular distress (that is, damage-associated molecular patterns) as a consequence of infection. In the case of NLRP3, several models that are not mutually exclusive have been discussed whereby activation of the NLRP3 inflammasome is not mediated by direct interaction between NLRP3 and the activating damage-associated molecular patterns. Instead, it is the result of substantial imbalances in the concentration of intracellular ions or reactive oxygen species (ROS) or the release of lysosomal content into the cytoplasm[8,9]. That in turn leads to assembly of the NLRP3 inflammasome via proteins such as thioredoxin-interacting protein or other yet-to-be-determined mediators[9]. Although NLRC4 was originally proposed to be a direct sensor of intracellular flagellin, subsequent studies have suggested a similar indirect activation model[10,11]. Members of the Naip subfamily of NLRs instead function as sensors of flagellin or moieties of the rod structure in the type III secretion system (PrgJ), which in turn physically interact with NLRC4, inducing the assembly of an inflammasome and a protective immune response[10,11]. Nonetheless, the dependence on NLRC4 for the activation of caspase-1 by some but not all flagellated, secretion system–containing bacteria raises the possibility that other unidentified bacteria-specific factors have regulatory

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roles in inflammasome activation. The identification of these host-evasion factors is expected to greatly enhance the knowledge of inflammasome function and regulation. Likewise, the mechanisms for the sensing of bacterial muramyl dipeptide or anthrax lethal factor by NLRP1 and of the microbial cytosolic signals that activate the NLRP3 inflammasome remain to be determined.

A second emerging concept relates to the complex and physiologically relevant cooperative interactions between the various inflammasomes during infection. Many bacterial pathogens, including (among others) *Salmonella, Shigella* and *Listeria*, activate the NLRC4 and NLRP3 inflammasomes. Notably, mice lacking both inflammasomes are more susceptible to *Salmonella* infection than are mice with single deficiency in NLRC4 or NLRP3 (ref. 12). Equally interesting are the cooperative roles of inflammasomes and other pathways of innate immunity. For example, the NLRC4 inflammasome and signaling by Toll-like receptor 5 have nonredundant roles in protection against infection with pathogens such as *Salmonella*, whereas the NLRP3-independent role of the RNA helicase RIG-I in activating caspase-1 contributes to the antiviral protective response[13]. Thus, it seems that in the *in vivo* setting, the antimicrobial innate host response involves complex redundant and complementary interactions between various inflammasomes and other innate microbe-sensing systems. The molecular nature of such interactions, which remains mostly unknown, is expected to take center stage in years to come.

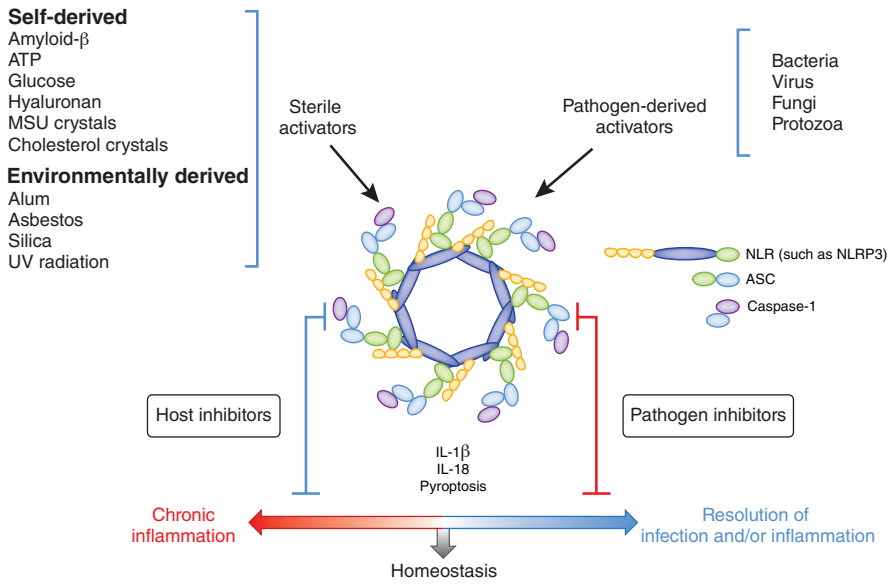

Figure 1 Inflammasome activity regulates homeostatic processes and inflammation during infection and tissue injury. During infection or injury, inflammasomes are directly or indirectly activated by a wide array of danger-associated molecular patterns. The initial event leads to activation of caspase-1, release of IL-1β and IL-18, and sometimes pyroptosis. Release of IL-1β and IL-18 results in recruitment of effector cell populations of the immune response and tissue repair. Under normal circumstances, activation of the inflammasomes culminates in the resolution of infection or inflammation and contributes to homeostatic processes (that is, intestinal microbial ecology and regeneration of epithelial cells after injury). However, perpetuation of inflammasome activation can lead to chronic inflammatory diseases. Pathogen-derived inhibitors block inflammasome activation and thus the resolution of infection, whereas host-derived inflammasome inhibitors prevent the perpetuation of chronic inflammation. UV, ultraviolet.

Regulation of inflammasomes

Mutations in genes encoding members of the inflammasome pathway, including NLRP3 itself and the phosphatase-interacting protein PSTPIP1, have been identified as driving forces in several autoinflammatory diseases in humans, which emphasizes the potential danger associated with mutations in genes encoding molecules involved in this pathway[14]. Notably, in many of these conditions, as well as in a spectrum of spontaneous inflammatory conditions, the clinical efficacy of therapies that target IL-1α and/or IL-1β has been proven, which suggests that tight control of the inflammasome is needed to prevent detrimental effects for the host. The review by Fitzgerald and colleagues in this issue discusses the multitude of intracellular and extracellular pathways that have been identified as contributing to the regulation of inflammasome activity[4]. These pathways allow rapid activation of inflammasome signaling after an insult to the host and subsequently downregulate inflammasome activity to prevent chronic inflammation.

Although some of the pathways described above act as nonspecific regulators of inflammasome signaling by inhibiting caspase-1 function, others are specific regulators of a particular inflammasome. A prominent example that has received much attention is the crosstalk between activation of the NLRP3 inflammasome and autophagy, a catabolic process able to degrade organelles and other large structures that has diverse functions in host defense[15]. It has been proposed that autophagy, which is induced by cellular stress, prevents the accumulation of activators of the NLRP3 inflammasome such as ROS and mitochondrial DNA in the cytoplasm and thereby limits inflammasome activation. However, the details of the interaction between these two pathways are still being debated intensely and future research will need to address the molecular nature of this important link. Immune pathways not only intersect within cells to regulate inflammasome signaling but also communicate with each other via extracellular mediators such as type I and type II interferons. Notably, their effects seem to be dependent on cell type and context, as both inhibitory and stimulating functions have been noted. Hence, the diverse layers that have evolved in organisms for the tight regulation of inflammasome activation support the proposal of a key role for this complex in protecting the host's integrity.

A common mechanism used by bacteria and viruses to evade the immune response involves molecules that interfere with the regulatory network aimed at clearing them from the host. Of no exception are the many microbe-expressed inhibitors of inflammasome activation aimed at curtailing the antimicrobial immune responses. However, another aspect of the host-microbe interaction that may have a broad effect is regulation of the expression of inflammasome-associated genes such as *Il1b* and *Il18* by commensal microbes[16]. It has been noted that as-yet-unidentified commensal bacteria that are sensitive to the antibiotic neomycin provide basal activation signals required for the expression of pro-IL-1β and pro-IL-18 in the lung. In their absence, mice show greater sensitivity to infection with influenza virus due to the lack of local inflammasome responses. Strikingly, this requirement is restricted to host responses that require inflammasomes but not to those that do not require inflammasome signaling.

Another example of a complex regulatory role of inflammasomes has been observed in

the intestine. The regulation of microflora in mice seems to be regulated by constitutive release of IL-18 by the nonhematopoietic intestinal compartment[17]. Deficiency in components of the NLRP6 inflammasome has been found to elicit a dysbiotic colitogenic microflora, dominated by the presence of the family Prevotellaceae and candidate phylum TM7; those organisms in turn are associated with enhanced susceptibility to colonic autoinflammation in NLRP6 inflammasome–deficient mice. The IL-18-dependent and IL-18-independent mechanisms by which various inflammasomes participate in the regulation of mucosal homeostasis, including the composition of the microbiome and tissue regeneration and repair, remain not well known. Finally, the composition of the intestinal microbiota varies considerably among people and hence we are tempted to speculate that crosstalk between microbes and inflammasomes regulates human local and systemic inflammasome-dependent immunity. In summary, detailed knowledge of the regulatory pathways involved in inflammasome signaling is needed to harness the potential that drugs targeting this important molecular complex offer for the treatment of inflammation-driven diseases in humans.

Regulators of metabolic disorders

Metabolic syndrome is associated with multiorgan inflammatory abnormalities (involving pancreatic, adipose, hepatic, cardiac and muscle tissue) and represents a major disease burden in the developed world. IL-1β is postulated to have a negative role in the pathogenesis of many metabolic disorders. Indeed, studies have tested recombinant IL-1 receptor antagonist for the treatment of patients with type 2 diabetes, with some encouraging results[18]. In contrast, IL-18-deficient mice are prone to develop obesity, hyperphagia and insulin resistance[19]. Despite the opposing roles of these proinflammatory cytokines, which most probably reflect their hierarchical contributions to different metabolic processes and tissues, these observations suggest that inflammasomes sense and respond to the metabolic status of an organism. How do inflammasomes sense metabolic cues in complex and multiorgan disorders?

A wealth of evidence has begun to shed some light into that question, indicating a close relationship between the NLRP3 inflammasome and the metabolism of lipids and carbohydrates. In adipose tissue, higher concentrations of saturated fatty acids such as palmitate and ceramides lead to lower activity of the kinase AMPK, which results in defective autophagy of mitochondria and therefore the accumulation of ROS in the cytosol; this in turn promotes NLRP3 activation and IL-1β release[20,21]. Higher concentrations of IL-1β promote insulin resistance and a proinflammatory effector T helper type 1 cellular infiltrate in adipose tissue. In pancreas, the deposition of fibrils of islet amyloid polypeptide and the intracellular accumulation of ROS due to hyperglycemia activate the NLRP3 inflammasome and promote the release of IL-1β from macrophages and beta cells[9,22], respectively. IL-1β then causes beta-cell dysfunction and death, which leads to less secretion of insulin. Consistent with that, NLRP3-deficient mice show better glucose tolerance and insulin sensitivity and seem to be protected from obesity induced by a high-fat diet. Finally, activation of the NLRP3 inflammasome by cholesterol crystals and IL-1β release has been associated with larger atherosclerotic lesions[23]. However, the concept that inflammasomes are only proinflammatory drivers through IL-1β release during nutritional surplus might just be too simplistic.

Caspase-1 cleaves enzymes of the glycolytic pathway but, perhaps more interestingly, caspase-1-deficient precursor cells differentiate more efficiently into mature adipocytes and have a higher oxidation rate than do wild-type precursor cells[24], which suggests that inflammasomes might regulate developmental programs in adipocytes and directly control cellular energy-metabolism pathways. Furthermore, the protection from obesity and atherosclerosis induced by a high-fat diet in an NLRP3-deficient setting has not been uniformly confirmed[25], which suggests that, among another reasons, differences in the gut microflora or housing conditions could influence some metabolic outcomes. Indeed, it has been demonstrated that dysbiosis induced by deficiency in the NLRP3 or NLRP6 inflammasome drives obesity and, coupled with enhanced hepatic Toll-like receptor signaling, drives the exacerbation of nonalcoholic fatty liver disease, which links inflammasome-microflora dysregulation with downstream metabolic consequences[26].

As discussed in the accompanying review by O'Neill and colleagues[5], the important regulatory functions of inflammasomes in the context of both metabolic homeostasis and metabolic abnormalities are only beginning to be elucidated. What are the roles of other NLR or PYHIN proteins in the pathophysiology of obesity? What is the nature of the stimuli that activate caspase-1 during adipocyte differentiation? What is the effect of inflammasome-mediated intestinal dysbiosis on metabolic processes? These are some of the exciting open questions that should be resolved in the coming years.

The inflammasome and carcinogenesis

Equally intriguing and likewise confusing is the participation of inflammasomes in tumor formation, progression and interaction with the microenvironment, as well as the antitumor inflammatory response. The review by Kroemer and colleagues discusses the complex and often contradictory contributions of different inflammasomes that are noted in almost all steps of tumor development[6]. Impaired pyroptosis, a hallmark inflammasome effector mechanism of cell death, has been suggested to be linked with a propensity for inflammation-induced development of colonic carcinoma in mice deficient in NLRP3 or NLRC4 inflammasome[27,28]. In contrast, the IL-1 axis has been shown to promote tumorigenesis through tumor cell–autonomous effects and through the generation of a favorable tumor niche by enhanced recruitment of suppressor cells of the immune response[29]. Moreover, IL-18 impairs the antitumor function of natural killer cells, thereby shielding the tumor from the host immune response[30].

Adding to the complexities noted above, inflammasome signaling in dendritic cells by products released from stressed tumor cells has been found to be crucial for the development of adaptive immune responses[31]. Deficiency in various inflammasome components or the administration of compounds that suppress the IL-1 axis is associated with high metastatic potential and poor response to chemotherapy. Thus, the beneficial effects of the IL-1 receptor antagonist anakinra on tumor development and progression, which has now been documented in many experimental models, might be offset by the harmful effects on the antineoplastic immune response elicited by chemotherapy or tumor vaccination. Furthermore, for mucosal surfaces such as the intestines, the striking effects of inflammasome deficiency on tissue homeostasis and repair and the composition of the microbiome may have fundamental effects on the propensity to develop inflammation-induced cancer, which adds yet another level of complexity to the inflammasome-mediated regulation of tumor development[32]. As in the infectious and metabolic scenarios, we believe that complex and cooperative effects mediated by different inflammasomes expressed by various cells of the tumor or surrounding non-tumor tissue at different stages of disease may determine the net effects of inflammasome signaling on tumor development. Elucidating the roles of individual NLR proteins and their upstream activators and modulators in various stages of

tumor development may enable the recognition of novel therapeutic targets for effective yet safe antineoplastic treatments.

Concluding remarks

The discovery of the inflammasome has generated an exciting new field of immunology. Inflammasome activation is now recognized as being critical in the host response to microorganisms and damage-associated molecular patterns. Moreover, the roles of inflammasomes have been extended to carcinogenesis, autoimmune disorders and metabolic syndrome. Despite this wealth of information, the exact molecular mechanisms by which some NLR inflammasomes are activated remain unresolved; furthermore, it is still unclear how cells 'decide' to engage death pathways after inflammasome activation. Finally, we expect that studies of mice with conditional inactivation of the various inflammasome components will identify previously unknown and essential tissue-specific functions for these vital platforms. This issue of *Nature Immunology* deservedly highlights the most important aspects of this rapidly expanding field.

AUTHOR CONTRIBUTIONS

J.H.-M., E.E., T.S. and R.A.F. contributed equally to this work.

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