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Inflammasomes and Metabolic Disease

Jorge Henao-Mejia,[1,*] Eran Elinav,[2,*] Christoph A. Thaiss,[2,*] and Richard A. Flavell[1]

[1]Department of Immunobiology, Yale University School of Medicine, New Haven, Connecticut 06520; email: richard.flavell@yale.edu

[2]Immunology Department, Weizmann Institute of Science, Rehovot, Israel 70100

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*These authors contributed equally.

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Abstract

Innate immune response pathways and metabolic pathways are evolutionarily conserved throughout species and are fundamental to survival. As such, the regulation of whole-body and cellular metabolism is intimately integrated with immune responses. However, the introduction of new variables to this delicate evolutionarily conserved physiological interaction can lead to deleterious consequences for organisms as a result of inappropriate immune responses. In recent decades, the prevalence and incidence of metabolic diseases associated with obesity have dramatically increased worldwide. As a recently acquired human characteristic, obesity has exposed the critical role of innate immune pathways in multiple metabolic pathophysiological processes. Here, we review recent evidence that highlights inflammasomes as critical sensors of metabolic perturbations in multiple tissues and their role in the progression of highly prevalent metabolic diseases.

INTRODUCTION

Multicellular organisms in general and mammals in particular have coevolved with a microbial world, creating the challenge of maintaining homeostatic mutualism with the commensal microflora while initiating protective immune responses against invading pathogens. This fundamental challenge is met by the microbe-sensing capabilities of the innate immune system. Several families of pattern-recognition receptors (PRRs) have evolved to recognize conserved microbial structures indicative of the presence of bacteria, viruses, parasites, or fungi (1). These microbe-associated molecular patterns (MAMPs) are vital for microbial growth and function and are therefore conserved across microbial phyla and stable during evolution. Examples of MAMPs include components of the bacterial cell wall, such as lipopolysaccharide (LPS) and peptidoglycan, as well as microbial nucleic acids. MAMPs involved in the recognition of multicellular parasites remain largely unknown. PRRs are located either in the cytosol, on the plasma membrane, or in endosomal compartments. Prototypic families of PRRs include the Toll-like receptors (TLRs), C-type lectin receptors (CTLs), RIG-I-like receptors (RLRs), and NOD-like receptors (NLRs). In the presence of a specific microbial ligand, these PRRs trigger a downstream signaling cascade, which leads to the activation of transcription factors and to the production of proinflammatory cytokines. These cytokines orchestrate the switch from tissue homeostasis to a state of inflammation that is aimed at removing the trigger of PRR signaling and restoring normal tissue function (2). Interestingly, many PRRs can be triggered not only by MAMPs, but also by host-derived signals that are indicative of tissue malfunction; these signals are collectively termed damage-associated molecular patterns (DAMPs). DAMP recognition by PRRs leads to a similar activation of downstream signaling cascades and to the production of proinflammatory cytokines. Examples of DAMPs include molecules that are usually located intracellularly, such as ATP and HMGB1, as well as proteins derived from the extracellular matrix, such as hyaluronan fragments.

As sensors of both PAMPs and DAMPs, the NLR family stands out due to the large variety of both endogenous and exogenous triggers that lead to the activation of its members (3). NLRs belong to a group of cytosolic receptor proteins of conserved domain structure. A subset of NLRs is capable of forming, under tightly regulated conditions, a multiprotein complex termed the inflammasome (4). Upon activation, these NLRs assemble multiple proteins to form speck-like aggregates in the cytosol. In addition to NLRs, the PYHIN family member absent in melanoma 2 (AIM2) is also capable of initiating inflammasome formation. The core function of the inflammasome is the recruitment and activation of proinflammatory caspases, resulting in the cleavage of the precursors of the cytokines IL-1β and IL-18 into their bioactive forms and in the initiation of a form of cell death termed pyroptosis. IL-1β and IL-18 are potent proinflammatory cytokines that exert a wide range of functions in inflammation, in the immune response against infection, and in the maintenance of tissue integrity.

Although a pivotal response to infection and tissue injury, inflammation has also been associated with many pathological processes. Overt acute inflammation leads to tissue damage, and nonresolving inflammation causes chronic tissue malfunction, suggesting an evolutionary trade-off between the rapid and effective response to perturbations in tissue homeostasis and the collateral damage on tissue function. In particular, many modern metabolic diseases, ranging from obesity and type 2 diabetes mellitus (T2DM) to atherosclerosis, pulmonary hypertension, and chronic liver disease, have been associated with a chronic inflammatory state (5). All these conditions are manifestations of the so-called metabolic syndrome, the most common endocrine disorder worldwide. Due to the relatively recent appearance of these disorders in mere decades rather than the millennia over which humans have evolved, as well as the generally late onset of the disease in the life of an affected individual, it is conceivable that inflammation-induced metabolic disease has

escaped the selection process of evolution and is becoming more apparent with increased life expectancy and the Western lifestyle. The link between PRR-triggered inflammation in response to (*a*) loss of tissue function caused by either infection or tissue injury and (*b*) aberrations in metabolic homeostasis may therefore hold the key to understanding and treating system-wide manifestations of the metabolic syndrome.

In this review, we highlight the role of inflammasomes in metabolic disease. First suggested as a sensor of metabolic stress by the late Jürg Tschopp and colleagues (6), inflammasomes have recently emerged as central orchestrators of metabolic function in health and disease (7). Remarkably, both aberrantly high inflammasome activation and abrogation of inflammasome signaling in different tissues lead to the development of metabolic disease, indicating the necessity for a finely balanced signaling cascade and downstream cytokine release. In the following sections, we provide an overview of the currently known inflammasomes, their activators and downstream effects, and their function in the context of a cellular surveillance system. We then discuss the role of inflammasome signaling on metabolic processes and its involvement in the molecular etiology of the metabolic syndrome.

TRIGGERS OF INFLAMMASOME SIGNALING

So far, at least five NLR family members—NLRP1, NLRP3, NLRP6, NLRP7, and NLRC4—and two PYHIN family members—AIM2 and IFI16—have been demonstrated to form an inflammasome (**Figure 1**). They differ in their requirement to recruit the adaptor protein ASC (apoptosis-associated speck-like protein), which contains a caspase activation and recruitment domain. Due to the dramatic consequences of inflammasome signaling—the production of some of the most potent proinflammatory cytokines as well as the induction of cell death—inflammasome activity needs to be tightly regulated. This regulation occurs at multiple levels and is best known for the NLRP3 inflammasome. First, the expression of the upstream NLR sensor protein is regulated at the transcriptional level. Thus, expression levels of NLRP3 are usually low in most cell types in the steady state, and the gene is transcribed in response to a stimulus termed signal 1 (8, 9). Signal 1 can be provided by ligands of other PRRs, by cytokines, or by reactive oxygen species (ROS) (10). In addition, posttranscriptional mechanisms regulate inflammasome signaling. For instance, the cell may express proteins inhibiting caspase-1 or proteins sequestering inflammasome components in the cytosol (11). Furthermore, the subcellular localization of inflammasome components may play a regulatory role by determining the availability of these components for inflammasome assembly (3).

Most importantly, inflammasome formation depends on activation by specific triggers, which are referred to as signal 2. The range of potential inflammasome stimulators that have been discovered over the past decade is impressive; such stimulators include microbe-derived substances, molecules related to endogenous damage, and environmental particles. Notably, the mechanisms by which these various stimuli induce inflammasome assembly remain almost entirely unknown for most inflammasomes. In the following subsection, we provide an overview of the currently known inflammasome activators and highlight some of the common themes that may underlie inflammasome assembly in response to these triggers.

Microbial Activators

Inflammasomes play a central role in the antimicrobial innate immune responses (12). Inflammasome activation can be detected after a large variety of pathogenic infections that can be bacterial, viral, fungal, or parasitic, and inflammasome-deficient mice show increased susceptibility in a

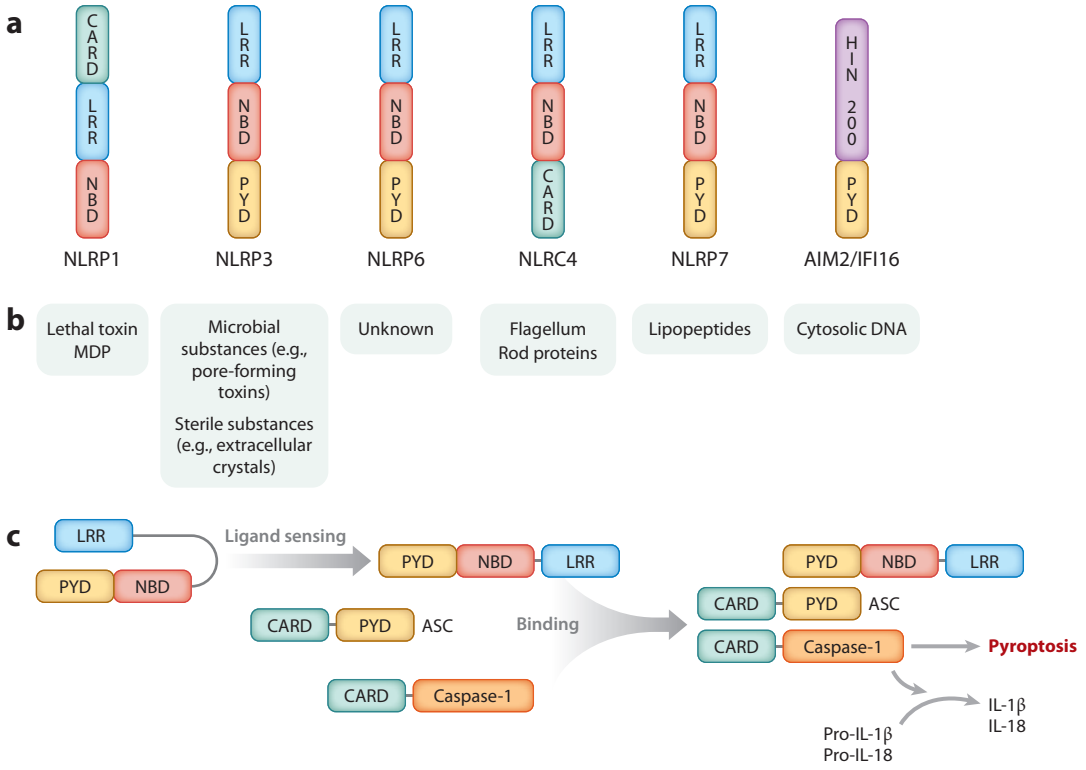

Figure 1

Inflammasome configuration and assembly. (*a*) Domain structure of the sensor NLR proteins currently known to form active inflammasomes. (*b*) Known activators of inflammasome formation. (*c*) A suggested activation scheme for inflammasome formation. The inactive form of the sensor NLR protein is folded upon itself in an autoinhibitory configuration. Upon ligand sensing, the autoinhibitory loop disengages, allowing the NLR protein to bind its inflammasome-forming partners and thus resulting in autocleavage and activation of caspase-1. Matured inflammasomes induce activation by cleavage of the cytokines IL-1β and IL-18 and mediate a proinflammatory form of programmed cell death named pyroptosis. Abbreviations: AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein; LRR, leucine-rich repeat; MDP, muramyl dipeptide; NBD, nucleotide-binding domain; NLR, NOD-like receptor; PYD, pyrin domain.

large number of animal models of infectious disease. However, in contrast to the situation for other PRRs, inflammasome activation by direct recognition of a microbial substance has been documented only in a minority of cases (13). Rather, inflammasome assembly may be triggered by secondary effects of pathogenic infection on essential cellular processes.

Direct recognition. NLRP1 initiates the formation of an inflammasome in response to *Bacillus anthracis* infection and can act as a sensor of anthrax lethal protein or muramyl dipeptide (MDP), a component of the bacterial cell wall, potentially suggesting direct recognition of microbial products by an NLR protein. In vitro, NLRP1 directly binds to MDP. However, another MDP sensor, NOD2, may be an important component of the NLRP1 inflammasome, indicating a role for other NLR proteins as cofactors in microbial recognition (14, 15). Such recognition mediated by bridging NLR proteins has also been described for NLRC4. Bacterial type III and type IV secretion systems activate the NLRC4 inflammasome. On the one hand, these secretion systems facilitate the translocation of the NLRC4 ligand flagellin into the cytoplasm, where flagellin recognition is

mediated by the NLR family member NAIP5, leading to NLRC4 inflammasome assembly. On the other hand, another NLR protein, NAIP2, facilitates the recognition of the type III secretion system rod protein PrgJ and thus initiates NLRC4 inflammasome activation (16, 17). Different NLRs therefore cooperate in recognition of microbial structures and create a more versatile repertoire for microbial ligand recognition upstream of inflammasome complex assembly. The non-NLR inflammasome activator AIM2 recognizes bacterial and viral double-stranded DNA and plays an important role in the antimicrobial response to intracellular pathogens, including DNA viruses and *Listeria monocytogenes* (18). The recently described NLRP7 inflammasome recognizes microbial acylated lipopeptides and signals through ASC and caspase-1 (19).

Indirect recognition. In most other cases, the structural diversity of inflammasome stimulators has led to the concept that, rather than serving as direct receptors for microbial products, NLRs serve as downstream assembly blocks for receptor proteins that recognize disturbances in cellular processes. This concept is further detailed below. In the case of NLRP3, several not mutually exclusive models suggest that distinct microbial signals mechanistically converge to result in (*a*) imbalances in the concentration of intracellular ions, (*b*) ROS production, or (*c*) the release of lysosomal content into the cytoplasm (20). A number of common themes in the characteristics of microbial NLRP3 activators have emerged. One important characteristic seems to be the capability of bacterial proteins to induce membrane instability by pore formation (21). The NLRP3 inflammasome is also activated by viral infection. Upon infection with influenza A virus, TLR7 signaling enhances transcription of NLRP3 inflammasome constituents. The influenza-encoded ion channel protein M2 causes potassium efflux and ROS production by pH neutralization of the *trans*-Golgi network, thereby activating the NLRP3 inflammasome (22). ROS production is also critical for NLRP3- and AIM2-mediated inflammasome activation in response to *Brucella abortus* infection (23). In addition, bacterial mRNA activates the NLRP3 inflammasome through TRIF [Toll/interleukin-1 receptor (TIR) domain–containing adaptor inducing interferon-β] (24).

Endogenous Activators

A remarkable feature of inflammasome signaling is that, in addition to acting as sensors of microbial components, endogenous DAMPs can act as inflammasome stimuli. The discovery that such DAMPs provide a signal for inflammasome assembly has in many cases provided the missing link between sterile tissue injury and the release of inflammatory mediators. The intracellular molecule ATP is released by damaged cells in many conditions of tissue injury, such as necrosis (25). Extracellular ATP is a trigger of the NLRP3 inflammasome by way of signaling through the $P2X_7$ receptor (26, 27). As such, ATP is an indicator of tissue integrity and can instigate sterile inflammation through the NLRP3 inflammasome.

Recently, investigators found that liposomes also activate the inflammasome through a mechanism dependent on calcium influx via the TRPM2 channel (28). Furthermore, oxidized mitochondrial DNA can endogenously activate the NLRP3 inflammasome during apoptosis (2). Interestingly, LPS-induced succinate production was recently identified as a proinflammatory metabolic process that enhances IL-1β production during inflammation (29).

In addition, crystalline molecules are activators of the NLRP3 inflammasome and lead to the production of IL-1β and IL-18. Extracellular precipitation and formation of monosodium urate (MSU) and calcium phosphate crystals result in phagocytosis by macrophages, during which these crystals activate the NLRP3 inflammasome through mechanisms involving lysosomal destabilization and ROS production (30). This process underlies the molecular etiology of gout, and mice lacking Nlrp3 are protected from MSU-induced neutrophil infiltration and inflammation (31).

The first clinical trials of blockers of IL-1β signaling in patients with gout yielded early promising results (32). Similarly, uric acid crystals, calcium pyrophosphate and oxalate crystals (affecting kidney disease) (33, 34), and hydroxyapatite crystals activate the NLRP3 inflammasome, and their levels in synovial fluid positively correlate with disease-promoting IL-1β levels in patients with osteoarthritis (35–37). Future clinical trials are needed to generalize the concept of inhibiting cytokine signaling downstream of inflammasome activation in patients with crystal deposition disorders. Although inflammasome activation in response to the release of molecules associated with loss of cellular integrity, such as ATP, may be an early alarm signal in response to tissue injury, the physiological importance of inducing inflammasome activation and downstream cytokine production in response to the deposition of endogenous crystalline substances may seem less apparent, highlighting the centrality of lysosomal destabilization in this process. A special example of inflammasome activation by endogenous activators is NLRP3 activation by amyloid β (38), which is enhanced in Alzheimer's disease and contributes to inflammatory pathology (39).

Environmental Activators

Consistent with the notion that crystalline substances activate the inflammasome, several environmental substances that form crystals have been implicated in aberrant triggering of NLRP3 inflammasome formation and ensuing pathologies. Examples of such noxious environmental inflammasome activators are silica and asbestos, which are phagocytosed by pulmonary macrophages; silica and asbestos activate the inflammasome through ROS production and lysosomal rupture, leading to silicosis and asbestosis, respectively (40–42).

Interestingly, the activation of the NLRP3 inflammasome by exogenous crystals also seems to have beneficial effects. Alum, the most commonly used adjuvant in human adjuvants, consists of aluminum hydroxide crystals that activate caspase-1 in an NLRP3-dependent manner in macrophages (43, 44). Nlrp3-deficient mice exhibit diminished alum-induced vaccine responses (45); other studies found the vaccination effect of alum to be dependent on ASC, but not on caspase-1 (46). A more recent study demonstrated that alum induced inflammasome-independent abortive phagocytosis in dendritic cells, leading to a humoral immune response (47).

In summary, the structural diversity of stimuli leading to inflammasome assembly, in particular the ones triggering NLRP3 inflammasome signaling, is a unique feature of inflammasomes among other PRRs, most of which are triggered by a more confined range of structural motifs. How these diverse signals converge into common downstream mechanisms that are responsible for inflammasome assembly still remains for the most part elusive. The discovery of such mechanisms will yield interesting insights into the biochemistry of inflammasome signaling and will open new avenues for the therapeutic intervention and manipulation of IL-1β and IL-18 production.

CONSEQUENCES OF INFLAMMASOME SIGNALING

The leucine-rich repeat (LRR) domain of most NLR family members is thought to play an autoinhibitory role (**Figure 1**). Upon stimulation with the appropriate upstream activating signal, this autoinhibition is released, and the nucleotide-binding domain (NBD) mediates homo-oligomerization or hetero-oligomerization, the first step in inflammasome assembly. Via homotypic PYD (pyrin domain) interactions, some NLR proteins recruit the adaptor molecule ASC, whereas some inflammasomes function independently of ASC. Subsequently, either the upstream NLR protein or the adaptor ASC recruits caspase-1 via homotypic CARD interactions. Assembly of multiple molecules of caspase-1 leads to autocatalytic cleavage and activation of this serine

protease. Active caspase-1 is the quintessential inflammasome effector molecule that is essential for its major functions: nonconventional protein secretion and pyroptosis (11).

How inflammasome component assembly is controlled has remained largely elusive. Interestingly, microtubules were recently found to play a role in this process (48). NLRP3 inflammasome activators reduce the intracellular concentration of the coenzyme NAD^+, thus inactivating the NAD^+-dependent tubulin deacetylase sirtuin-2. This inactivation in turn causes the accumulation of acetylated α-tubulin and apposition of mitochondria-located ASC to endoplasmic reticulum–located NLRP3. Similar to NLRC4 assembly, NLRP3 inflammasome assembly may be promoted by the recruitment of cofactors. Guanylate-binding protein 5 enhances NLRP3 inflammasome assembly in response to microbial, but not crystalline, substances (49).

Nonconventional Cytokine Secretion

IL-1β is one of the most potent proinflammatory cytokines and acts on a large variety of cell types and target tissues (50). It mediates the acute-phase response: fever, pain sensitivity, hypotension, and vasodilation. Corresponding to the large array of target tissues for IL-1β signaling, its receptor, IL-1RI, is almost ubiquitously expressed. IL-1RI shares features with other signaling receptors of the innate immune system, most prominently the TLRs. A TIR domain forms the cytoplasmic tail of the IL-1RI and recruits the adaptor protein MyD88, which in turn initiates signaling through the p38 mitogen-activated protein kinase, nuclear factor κB, and c-Jun N-terminal kinase pathways.

The potency of IL-1β necessitates tight control of its production. The cytokine is synthesized as a proprotein without significant biological activity and requires caspase-1 activation for release. Remarkably, unlike most other cytokines, IL-1β does not contain the typical amino-terminal signal sequence that would allow its translocation to the cellular secretion pathway. Similarly, IL-18, IL-1α, and fibroblast growth factor-2 do not harbor a leader sequence for endoplasmic reticulum/Golgi-mediated secretion but are instead released through a largely elusive process termed nonconventional protein secretion.

Two models explain how caspase-1-processed cytokines leave the cell once cleaved into their active forms. One model involves sequestration of the cytokine by secretory lysosomes. According to this model, caspase-1 and IL-1β are translocated into secretory lysosomes upon stimulation of the cell with microbial ligands and are eventually released from the cell by fusion of this compartment with the plasma membrane in response to a second trigger (signal 2), such as ATP (51, 52). However, the transporters and mechanisms involved in this process are unknown. Another hypothesis involves the shedding of microvesicles from the extracellular side of the plasma membrane. The formation of endosomal vesicles may accompany caspase-1 complex formation and enrich caspase-1 and IL-1 within the resulting multivesicular bodies (53, 54).

Pyroptosis

In addition to its role in nonconventional protein secretion, inflammasome activation also mediates the initiation of pyroptosis, an inflammatory form of cell death that depends on caspase-1 activity (55). Pyroptotic cells undergo DNA fragmentation, which is usually observed during apoptosis. However, unlike apoptosis, pyroptosis does not lead to membrane blebbing or to exposure of phosphatidyl serine (a so-called "eat-me" signal) for the recruitment of phagocytes. Instead, membrane integrity is lost during pyroptosis, a feature that is important for nonconventional protein secretion, as described above. Loss of membrane integrity also leads to observable cell swelling. Both pyroptosis and apoptosis require the activation of caspases; however, pyroptosis occurs independently of apoptotic caspases, including caspase-3, caspase-8, and caspase-9, whereas apoptosis

occurs in the absence of caspase-1. Very recently, however, researchers showed that AIM2 and NLRP3 inflammasome signaling activates caspase-8, demonstrating that inflammasome signaling can be involved in both apoptosis and pyroptosis (56). A further distinction can be made on the basis of chromatin changes during the different processes of cell death. In apoptosis, pyknosis, which involves chromatin condensation and localization to the nuclear membrane, occurs. In addition, the nucleus of apoptotic cells undergoes karyorrhexis, i.e., disassembly and breakup. Neither pyknosis nor karyorrhexis can be observed in pyroptotic cells. Instead, the nucleus remains intact during this form of inflammatory cell death (57, 58). Pyroptosis also exhibits similarities with necrosis, in particular cytokine release from dying cells and the elicitation of an inflammatory response. A common theme in cell death is that the assembly of a multiprotein complex initiates a downstream signaling cascade that executes the cell death program: the APAF1-containing apoptosome for apoptosis, NLR-mediated inflammasomes for pyroptosis, and the RIP3-dependent necrosome for necrosis (59, 60).

Pyroptosis was observed long before the discovery of the inflammasome. More than 20 years ago, macrophages infected with *Shigella flexneri* or *Salmonella enterica* were shown to exhibit pyroptosis, although the term was coined only approximately 10 years later (57, 61, 62). "Pyro" stems from the Greek word for fire, in reference to the ignition of inflammation and fever-inducing IL-1β during inflammasome activation. "Ptosis" stems from the Greek word for falling (leaves), as in apoptosis and necrosis. So far, pyroptosis has been noted only in macrophages and dendritic cells, and whether a similar process occurs in other cell types remains a very interesting question.

INFLAMMASOMES AS A SURVEILLANCE SYSTEM OF CELL FUNCTION

Our insights into the processes of nonconventional protein secretion and pyroptosis, both of which depend strictly on inflammasome signaling, suggest that inflammasome assembly has evolved as a cellular emergency alarm system. This emergency system potentially responds to conditions that are incompatible with cell survival to such an extent that the cell undergoes self-sacrifice to release very potent alarm signals to neighboring cells and into the systemic circulation. These alarm signals lack a leader sequence for secretion, meaning that they are normally strictly intracellular until the appearance of adverse conditions resulting in the release of these cytokines as part of the last-resort cellular response. This concept, however, is difficult to apply to cell types that are less dispensable than myeloid cells, in particular nonhematopoietic cells, in which inflammasome signaling has been documented. Whether these cells also undergo pyroptosis upon inflammasome assembly in vivo and which mechanisms determine cell specificity of inflammasome signaling remain intriguing questions.

Given the enormous structural diversity of inflammasome triggers, NLR protein–mediated inflammasome assembly is unlikely to follow a pattern of classical microbial ligand recognition and downstream signaling. Instead, an emerging concept is that NLRs are guardians of major cellular functions (4). Perturbations in these functions generate recognizable signals that lead to NLR activation, inflammasome assembly, and caspase-1 cleavage (**Figure 2**). Although in most circumstances these signals are yet to be determined, recurrent themes regarding the nature of these inflammasome triggers are loss in membrane integrity (e.g., through pore-forming toxins, lysosomal rupture, or leakage of organelle content into the cytoplasm) and changes in cellular redox homeostasis (e.g., aberrant ROS production). In addition to monitoring the function and integrity of the plasma membrane and the lysosome, inflammasomes seem to do the same for mitochondria, a third organelle vital for cellular function (63).

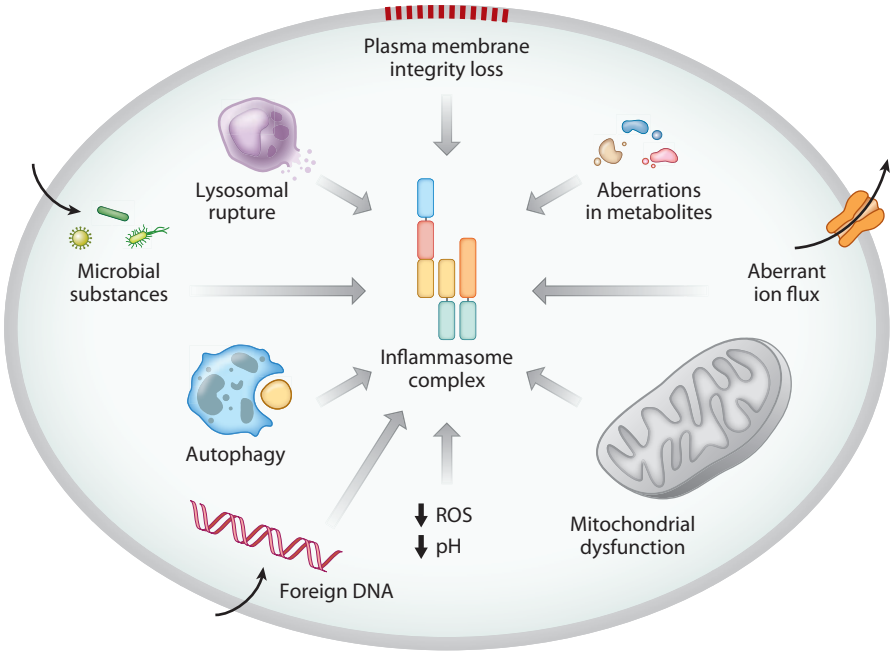

Figure 2

Model of an inflammasome surveillance system guarding vital cellular processes. Inflammasomes assemble in response to disruptions in various homeostatic cellular processes. These disruptions include loss of function of organelles (e.g., plasma membrane, lysosome, mitochondria, autophagosome), invasion of microbial products (e.g., effector proteins, nucleic acids), and aberrations in homeostatic set points of cellular processes (e.g., metabolites, redox homeostasis, pH). ROS denotes reactive oxygen species.

Because perturbations in cellular and tissue homeostasis that induce inflammasome activation are also pathognomonic features of tissues with metabolic abnormalities, it is not surprising that recent discoveries provide unequivocal evidence that inflammasomes are an essential surveillance system of the metabolic state of a cell and the whole organism. In the following section, we summarize the current knowledge about the involvement of inflammasome signaling in metabolic processes and highlight its role in the etiology of metabolic syndrome.

INFLAMMASOMES AND METABOLIC DISEASES

The incidence of obesity worldwide has increased dramatically in recent decades. In 2008, 35% of the world's adult population (1.4 billion people) were overweight, and 11% (500 million people) were obese; moreover, nearly 2.8 million adults die each year as a consequence of obesity-related disorders. Obesity is associated with multiorgan (e.g., cardiac, adipose, muscle, hypothalamic, pancreatic, and hepatic tissue), chronic metabolic and inflammatory alterations that together are termed metabolic syndrome. The risk of developing serious pathological conditions such as T2DM, nonalcoholic fatty liver disease (NAFLD), and cardiovascular disease is significantly associated with obesity and its concurrent multiorgan abnormalities. These pathologies represent a great burden upon Western societies, as they require significant resources from health care systems (64, 65). Thus, understanding the tissue-specific pathogenic processes that lead to disease progression is required for the development of more effective therapeutic approaches.

Intense research in the past decade on the pathogenic mechanisms that drive the progression of obesity-associated diseases has identified chronic low-grade inflammation (metabolic inflammation) as a pathognomonic feature associated with chronic nutritional surplus (64). Furthermore, metabolic inflammation is now considered to be a focal point in the initiation and progression of gout, insulin resistance, T2DM, atherosclerosis, and NAFLD. Thus, understanding the mechanisms that initiate inflammation in the context of these diseases is increasingly important for the understanding of the natural history of these serious illnesses.

As discussed above, the unique capability of inflammasomes to recognize a great variety of perturbations to intracellular and tissue homeostasis makes them ideal candidates to sense abnormal metabolic conditions in multiple tissues (3). Not surprisingly, unequivocal experimental evidence and clinical evidence have causally linked multiple inflammasomes, IL-1β, and IL-18 to the development of metabolic inflammation and to the progression of metabolic pathologies and their complications (66, 67). Nevertheless, the underlying molecular mechanisms that result in tissue-specific inflammasome activation in the context of obesity have just begun to be elucidated (**Figure 3**). In the following sections, we review recent findings on the role of inflammasome activation in the pathogenesis of obesity-associated pathologies and discuss the main challenges for the near future in this rapidly growing field.

Type 2 Diabetes Mellitus

T2DM is a chronic disease characterized by elevated levels of glucose as a consequence of peripheral insulin resistance accompanied by decreased pancreatic insulin secretion. In 2011, 25.8 million children and adults in the United States—8.3% of the population—were living with T2DM. Moreover, it is estimated that T2DM is the underlying cause or a contributing factor of 231,000 deaths per year, and the total costs of associated T2DM treatment in the United States reached $245 billion in 2012 (68). Thus, understanding the role of inflammation in the pathogenesis of this highly prevalent disease is essential for curtailing its devastating effects in Western societies.

The first link between obesity-associated metabolic inflammation and insulin sensitivity came from early studies that demonstrated that the levels of proinflammatory cytokines such as TNF-α, IL-6, and IL-1β were increased in the circulation and adipose tissue of obese and diabetic subjects (69–74). Subsequent studies using genetic mouse models demonstrated that proinflammatory cytokines inhibit insulin signaling by direct serine phosphorylation of insulin receptor substrate-1 (IRS1) (75, 76). Activated macrophages in adipose tissue are suggested to be the major source of proinflammatory cytokines. Populations of adipose tissue macrophages (ATMs) of obese individuals are composed mainly of classically activated proinflammatory M1 macrophages rather than composed of alternatively activated anti-inflammatory M2 macrophages. Moreover, the presence of these characteristic populations of ATMs is directly correlated with lipid ectopic accumulation and insulin sensitivity in multiple mouse models (65, 77, 78).

Accumulated evidence in the past decade indicates that members of the TLR family and inflammasomes expressed in ATMs sense metabolic disturbances or microbe-derived products in obese individuals. Such disturbances trigger the secretion of proinflammatory cytokines. The expression of TLR2 and TLR4, which provide signal 1 for inflammasome activation, is increased in adipose tissue of obese/diabetic individuals and in circulating monocytes of T2DM patients; furthermore, such expression correlates with the severity of insulin resistance (79, 80). The relevance of this correlation has been extensively studied in genetically modified rodent models. Interestingly, these studies have shown that loss of TLR4 function in the hematopoietic compartment reduces inflammation, levels of proinflammatory cytokines, and insulin resistance in adipose tissue

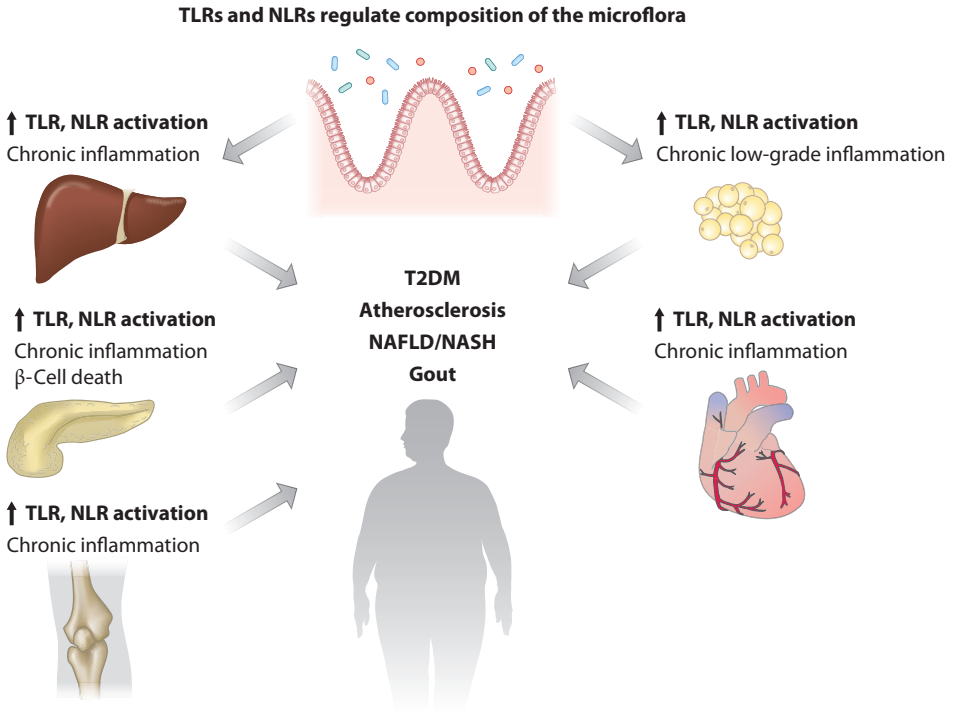

Figure 3

The role of inflammasomes in metabolic syndrome. During the pathogenesis of obesity-associated diseases, Toll-like receptors (TLRs) and inflammasomes are activated by damage-associated molecular patterns (DAMPs) associated with nutritional surplus in multiple tissues and cell types. This activation promotes the development of chronic low-grade inflammation, leading to the deterioration of metabolic functions. In the liver, TLR activation by endogenous DAMPs or translocated intestinal products leads to nonalcoholic fatty liver disease (NAFLD) progression and chronic inflammation. In adipose tissue, palmitate and ceramides activate the NLRP3 inflammasome in infiltrating macrophages, which leads to increased insulin resistance. In the pancreas, elevated levels of glucose and islet amyloid polypeptide (IAPP) deposition activate TLRs and inflammasomes. As a consequence, increased β-cell death and decreased insulin secretion occur. Cholesterol crystals in early atherosclerotic lesions activate the NLRP3 inflammasome in infiltrating macrophages, promoting inflammatory cell infiltration and increased atherosclerosis progression. In joints, crystal formation of monosodium urate (MSU) and calcium phosphate activates the NLRP3 inflammasome, promoting gout progression in an IL-1β-dependent manner. In addition, chronic low-grade inflammation during obesity is associated with increased gut permeability, whereas innate immune receptors regulate the composition of the intestinal microbiota. Other abbreviations: NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus.

(81, 82). Moreover, functional deficiency of TLR4 can reduce high-fat diet (HFD)-induced hepatic steatosis and inflammation, leading to improved hepatic insulin sensitivity (79, 83). Various in vitro as well as in vivo studies show that, as does TLR4, TLR2 mediates metabolic inflammation and insulin resistance in liver, muscle, and adipose tissue (79, 81).

The expression of the components of the NLRP3 inflammasome (NLRP3, ASC, and caspase-1) is increased in adipose tissue and livers of obese mice and humans; moreover, the expression level is directly correlated with the severity of T2DM in obese individuals (84), and the reduction of such expression in adipose tissue is associated with an improvement in insulin sensitivity

(84, 85). NLRP3, ASC, and caspase-1 are preferentially expressed in ATMs, where the saturated fatty acid palmitate and lipotoxic ceramides trigger NLRP3 inflammasome activation through a mechanism that involves defective autophagy and the accumulation of mitochondrial ROS (84, 86). Mice lacking caspase-1 or Nlrp3 develop reduced adiposity with attenuated adipose tissue inflammation and exhibit improved insulin sensitivity upon HFD (84, 86).

The elevated levels of NLRP3 inflammasome activation in ATMs of obese mice result in at least two deleterious consequences in insulin-sensitive tissues: (*a*) IL-1β secretion inhibits insulin signaling by direct serine phosphorylation of IRS1 and induces expression of TNF-α, a well-characterized insulin resistance–promoting cytokine (76, 87), and (*b*) IL-1β and IL-18 induce an effector Th1 proinflammatory profile in adipose tissue (84), which may worsen metabolic outcomes in obesity. In concordance with the deleterious effects on insulin signaling, IL-1β deficiency protects mice from HFD-induced insulin resistance (85, 86), and treatment with an IL-1 receptor antagonist (IL-1RA) reduces hyperglycemia in obese/diabetic rodents and improves glycemic control in T2DM patients (80, 88). Moreover, polymorphism in the genes encoding IL-18 and IL-1RA was associated with increased risk of metabolic abnormalities (89, 90).

In adipose tissue, inflammasomes may also have nonhematopoietic functions. Caspase-1 is upregulated during adipocyte differentiation, and its activation promotes IL-1β secretion and insulin resistance through autocrine effects. But perhaps more interestingly, caspase-$1^{-/-}$ adipocyte precursors differentiate to mature adipocytes more efficiently and have an increased oxidation rate (91), suggesting that adipocyte-specific proteins may serve as caspase-1 substrates, leading to metabolic changes in the adipocytes of obese animals. However, the nature of the sensor proteins that trigger adipocyte-specific caspase-1 activation remains to be elucidated.

A physiological response to chronically elevated glycemic levels as a consequence of insulin resistance is increased insulin secretion by pancreatic β cells. Chronic exposure to high levels of free fatty acids and glucose induces islet inflammation and results in increased apoptosis and impaired insulin secretion of β cells, which prompts the progression from obesity and insulin resistance to overt T2DM (80). Importantly, IL-1β seems to play a critical role in the pathological deterioration of pancreatic islet function. IL-1β is preferentially expressed in β cells and infiltrating macrophages (92). Moreover, human clinical trials using IL-1RA significantly improved β-cell function and glycemic control in T2DM patients (93).

Secretion of IL-1β in pancreatic islet failure during T2DM depends mainly on TLR4 signaling (signal 1) and NLRP3 inflammasome activation. Activation of TLR4 signaling triggered by elevated levels of pancreatic palmitate levels leads to β-cell dysfunction and promotes the recruitment of inflammatory monocytes to islets (94). The NLRP3 inflammasome is activated in pancreatic islets through two different mechanisms in a cell type–specific manner. First, the severity of T2DM closely correlates with high levels of deposition of islet amyloid polypeptide (IAPP; also known as amylin) within pancreatic islets (95). The accumulation of high levels of IAPP activates the NLRP3 inflammasome in infiltrating pancreatic macrophages that were previously primed (signal 1) with the abundant minimally oxidized low-density lipoprotein (mmLDL) through the disruption of lysosomes and increased ROS production (96). The relevance of this pathological activation is further supported by the significantly higher levels of IL-1β found in infiltrating pancreatic macrophages from human IAPP transgenic mice (96). Second, β cells chronically exposed to high levels of glucose have increased mitochondrial ROS production as a consequence of the enhanced activity of the electron transport chain, which in turn leads to the dissociation of thioredoxin-interacting protein from thioredoxin and thus to NLRP3 activation (97). Secreted IL-1β can then act in a paracrine or an autocrine manner to induce the expression of chemotactic molecules or β-cell death (96).

Great advances have been made in understanding the role of inflammasomes in islet function. Future research should address the exact contribution of cell-specific activation of inflammasomes during T2DM progression in the context of obesity and insulin resistance.

Atherosclerosis

The leading cause of mortality worldwide is cardiovascular disease (CVD). Approximately 16.7 million deaths occur each year, and it is expected that the incidence of this deadly disease will continue to rise steadily in the following decades, reaching 25 million deaths per year in the third decade of this century (98). The most common underlying cause of CVD is atherosclerosis, which is a condition of the wall of mid-size and large-size blood vessels that is caused by lipid-induced inflammation. As such, atherosclerosis is significantly associated with hyperlipidemia induced by obesity and accounts for 70% of morbidity of T2DM patients (90, 99). The pathogenesis of atherosclerosis is a process that requires a complex and orchestrated interaction between macrophages, endothelial cells, and smooth muscle cells. Although the inflammatory nature of atherosclerosis is well established, the biological agents that trigger artery wall inflammation and the innate immune receptors that sense perturbations in blood vessel homeostasis have just begun to be elucidated.

The initial stages of atherosclerosis are characterized by subendothelial retention of circulating LDL particles, which leads to their oxidative modification by ROS and enzymatic attacks. Subsequently, mmLDLs can activate TLRs, resulting in the rapid transcription of inflammasome-processed cytokines (e.g., IL-1β) as well as that of multiple other proinflammatory soluble factors such as chemokines, costimulatory molecules, and cytokines (100). The generation of this proinflammatory milieu results in the recruitment of myeloid cells and T cells into the intima, where monocytes differentiate into macrophages and then into foam cells as a consequence of cholesterol accumulation. Foam cells are a pathognomonic feature of atherosclerotic plaques and play a critical role in disease progression (100, 101).

From the TLR family, TLR2 and TLR4 play a critical role in atherosclerosis progression. In the hypercholesterolemic apolipoprotein E (ApoE)-deficient murine model, lack of MyD88, which is a common signal transduction molecule for most TLRs, decreases atherosclerosis development (102, 103). In concordance, TLR4 deficiency in mice attenuates vascular inflammation during diet-induced obesity and reduces aortic plaque burden in the ApoE knockout and LDL receptor (LDLR)-deficient murine models of atherosclerosis (102, 104–106). In addition, TLR2 deficiency reduces atherosclerosis severity in multiple models of atherosclerotic plaque formation (107, 108). The putative TLR4 and TLR2 ligands during atherosclerosis disease are mmLDL, oxidized LDL, fibronectin, heat shock proteins, and carboxyethylpyrrol (109, 110).

Plasma concentrations of both IL-1β and IL-18 are elevated in atherosclerosis, and such elevations are associated with disease severity (90), suggesting a critical role for inflammasomes in the etiology of atherosclerosis. Cholesterol crystal deposition in arterial vessels has long been known as a feature of atherosclerotic plaques; moreover, recent studies have shown that these crystals are present in the early stages of atherosclerotic lesions, coinciding with the first appearance of inflammatory cells (111). Importantly, cholesterol crystals disrupt the lysosome and activate the NLRP3 inflammasome in primed macrophages ex vivo (111). Strikingly, $LDLR^{-/-}$ mice reconstituted with NLRP3, ASC, or IL-1α/β-deficient bone marrow have a significant reduction in the progression of atherosclerosis, suggesting that NLRP3-inflammasome activation and IL-1 secretion from the hematopoietic compartment are the key parameters in the early stages of plaque formation (111). Similarly, recent studies showed that caspase-1 deficiency also reduces vascular inflammation and atherosclerosis progression in the $ApoE^{-/-}$ model (112, 113). In contrast,

earlier reports using ApoE-deficient mice in the context of deficiency of different components of the NLRP3 inflammasome showed that atherosclerosis progresses independently of the NLRP3 inflammasome (114). These discrepancies are mostly likely the consequence of different doses of cholesterol and durations of the atherogenic diet used in these studies. Although a clear picture has emerged as to the role of inflammasomes in the hematopoietic compartment in the pathogenesis of atherosclerosis, the role of different inflammasomes in nonhematopoietic cells during atherosclerotic plaque formation warrants further research.

Nonalcoholic Fatty Liver Disease

NAFLD is the main cause of liver pathology in Western societies and is considered to be the hepatic manifestation of metabolic syndrome. Its prevalence reaches 30% in the general population and up to 75–100% in obese individuals. Moreover, the incidence of NAFLD in children is rising dramatically in the United States as a consequence of the obesity epidemic. The prevalence of this disease is expected to reach 50% in the American population by 2030 (65). Different degrees of severity characterize this disease; although the great majority of patients with steatosis (the accumulation of lipids in hepatocytes) are asymptomatic, nearly 20% eventually progress to develop chronic hepatic inflammation [nonalcoholic steatohepatitis (NASH)], which in turn can lead to portal hypertension, cirrhosis, hepatocarcinoma, and increased mortality (115). NASH is classified into primary NASH (associated with obesity and hyperlipidemia) and secondary NASH (related to pharmacological interventions, Wilson's disease, or jejuno-ileal bypass surgery). Surprisingly, although NAFLD/NASH is one of the most prevalent metabolic aberrations in humans, the insults that trigger inflammation have remained elusive. However, great progress has recently been made in identifying the key innate immune receptors involved in disease progression (116).

A combination of hepatic insults is proposed to drive NAFLD/NASH pathogenesis. Hepatic steatosis predisposes the liver to additional proinflammatory insults, which in turn promote infiltration of the hepatic parenchyma by immune cells, leading to disease progression. Thus, in the context of hepatic steatosis, the parallel actions of increased generation of ROS, augmented lipid peroxidation, and intestine-derived factors are required for the development of steatohepatitis (116, 117). As each of these hepatic insults is known to activate a variety of innate immune pathways, a growing body of research now functionally links the innate immune system to NAFLD progression.

Various members of the TLR family are expressed in the liver. More specifically, Kupffer cells express high levels of TLR2, TLR4, and TLR9 (118, 119); likewise, liver sinusoidal endothelial cells respond to TLR1, -4, -6, -8, and -9 ligands. Not surprisingly, recent evidence indicates that signaling through TLRs is critical for the progression of NAFLD. Multiple reports in a variety of models have demonstrated that activation of TLR4 signaling has a deleterious impact on NAFLD progression (119), which is in accord with the significantly higher levels of LPS in serum of patients with NAFLD and with the finding that antibiotic treatment attenuates steatosis in these individuals (120). Furthermore, wild-type mice on a choline-deficient, amino acid–defined diet developed severe steatohepatitis and insulin resistance, whereas TLR9-deficient mice showed significantly lower levels of steatohepatitis, fibrogenic response, and insulin resistance (118). Thus, intrahepatic activation of TLR signaling by endogenous and pathogen-derived ligands plays a critical role in the development of NASH and is essential for inflammasome activity during liver pathologies.

Different inflammasome components are expressed in multiple cell types in the liver. For instance, hepatocytes upregulate NLRP3 expression upon activation of TLR4 signaling; moreover, Kupffer cells and sinusoidal endothelial cells express high levels of AIM2, NLRP1, and NLRP3 (121). Likewise, inflammasomes play a critical role in the regulation of intestinal microbiota

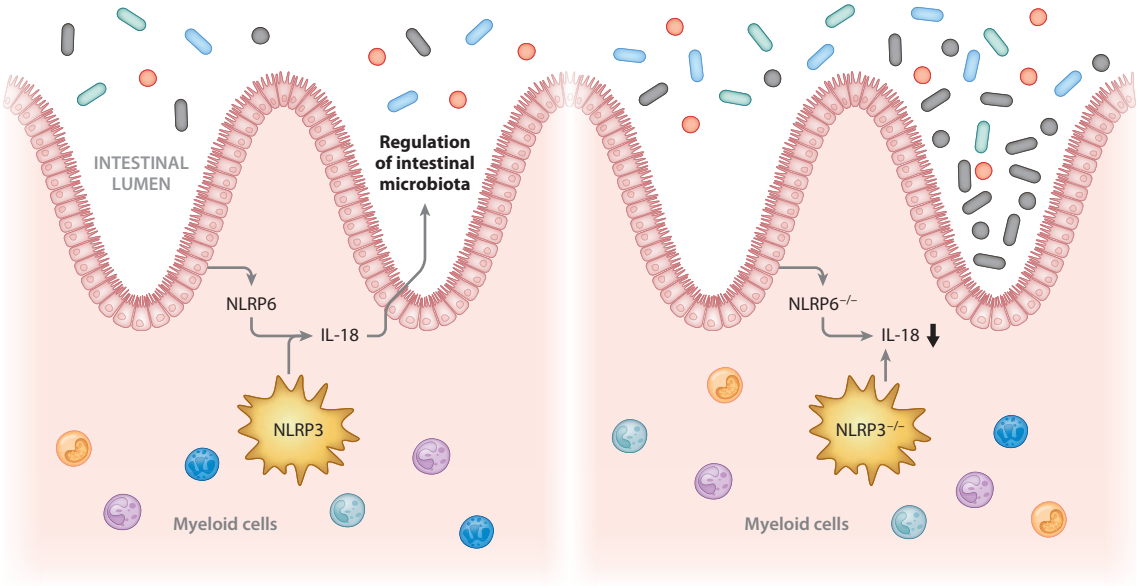

Figure 4

Regulation of the intestinal microbiota by inflammasomes. Activation of the NLRP6 inflammasome in colonic epithelium or the hematopoietic NLRP3 inflammasome in myeloid cells leads to constitutive IL-18 secretion in the colon. IL-18 regulates the composition of the intestinal microbiota through its activity in colonic epithelial cells. In the absence of the NLRP6 or NLRP3 inflammasomes, the levels of IL-18 are significantly decreased, leading to dysbiosis characterized by the expansion of *Prevotellaceae* and TM7.

composition, and their absence leads to the expansion of procolitogenic intestinal microbial communities (**Figure 4**) (122). Approximately 75% of hepatic blood flow (1,000–1,200 mL/min) is derived from the hepatic portal vein, and as such the liver is constantly exposed to antigens and microorganisms derived from the intestinal tract (123). Thus, the inflammasomes can have local and intestinal functions in the pathogenesis of NAFLD.

We recently reported that the aberrant microbiota in inflammasome-deficient mice has profound effects on NAFLD progression (124). Using the methionine-choline-deficient diet, HFD, and leptin receptor–deficient mouse models, we showed that mice carrying the inflammasome deficiency–associated procolitogenic flora develop more severe NAFLD/NASH, which is fully transferable to wild-type mice upon prolonged cohousing.

Enhanced liver inflammation is induced by increased levels of TNF-α as a consequence of TLR4 and TLR9 activation by TLR agonists carried to the liver in the portal circulation. In concordance with these findings in mouse models, recent reports indicate that patients with inflammatory bowel disease have significantly greater incidence of NAFLD; moreover, this incidence was reduced among patients who received anti-TNF-α therapy (125). Interestingly, the NLRP3 inflammasome may play a role in the local events that lead to NAFLD/NASH progression (126). Upon induction of NAFLD/NASH, activation of the inflammasome was detected in isolated hepatocytes where multiple components of these multiprotein complexes were also expressed. Mechanistically, palmitate activated the inflammasome in hepatocytes, leading to IL-1β secretion. In conclusion, in environmental settings in which a procolitogenic flora is expanded as a result of inflammasome deficiency, these bacterial communities may play a dominant role in disease progression. Nevertheless, hepatic activation of inflammasomes during NAFLD suggests

that their activation in the absence of dysbiosis may have an important role in the pathogenesis of this disease; this hypothesis warrants further investigation.

Gout

Gout is a disease associated with a diet rich in purines and with high concentrations of uric acid. The prevalence of gout in the United States has reached 4% in the general population (8.3 million Americans). Moreover, the prevalence of hyperuricemia has also increased, affecting 21% of the adult American population (127). The pathogenesis of gout is driven by the accumulation and deposition of uric acid crystals in the joints, and as such these crystals are potent triggers of inflammasome activation.

The pivotal role of inflammasomes in the etiology of gout is clearly demonstrated by clinical studies that show that blockade of IL-1β significantly reduces disease severity (32, 128–130). MSU crystals induce cleavage of caspase-1 in LPS-primed macrophages through an NLRP3- and ASC-dependent process (31). Furthermore, the centrality of the NLRP3 inflammasome in this process was demonstrated in vivo in a crystal-induced peritonitis model, whereby mice deficient in inflammasome pathway components exhibited markedly diminished peritonitis, and in a mouse model of gouty arthritis (131, 132).

FUTURE DIRECTIONS

Inflammation is a critical component of metabolic syndrome. Great progress has been made in the understanding of how proinflammatory cytokines and specific immune cell populations promote metabolic disease progression. However, our understanding of how the host immune system senses metabolic stress generated by nutritional surplus in vivo in a cell-specific manner still remains limited. Moreover, the exact contribution of those interactions to disease progression is still, for the most part, undetermined. The discovery of the inflammasomes as the cornerstone of the cytoplasmic surveillance system has led to important advances in our understanding of how the immune systems of mammalian organisms sense perturbations of cellular homeostasis. In the following years, it will be essential to understand how these multiprotein complexes sense a wide array of alterations in a cell type–specific manner and at which stage of metabolic disease these interactions impact disease progression. Our understanding of these processes will potentially lead to improved therapeutic approaches for treating these devastating diseases.

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