REVIEW

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Inflammasomes in health and disease

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Inflammasomes are a group of protein complexes built around several proteins, including NLRP3, NLRC4, AIM2 and NLRP6. Recognition of a diverse range of microbial, stress and damage signals by inflammasomes results in direct activation of caspase-1, which subsequently induces secretion of potent pro-inflammatory cytokines and a form of cell death called pyroptosis. Inflammasome-mediated processes are important during microbial infections and also in regulating both metabolic processes and mucosal immune responses. We review the functions of the different inflammasome complexes and discuss how aberrations in them are implicated in the pathogenesis of human diseases.

Inflammation is an acute response to infection and tissue damage to limit harm to the body[1]. However, dysregulated and chronic inflammation may result in secondary damage and immune pathology to the host. Inflammation is initiated on the sensing of signs of acute damage or disturbances of the steady state. Several recognition systems have evolved together to distinguish between homeostasis and threats to the host. Some of these receptors recognize distinct conserved pathogen-associated molecular patterns (PAMPs) that are predominantly found in microbes and hence allow the exquisite sensing of pathogens in tissues that are normally devoid of these structures[2]. Multiple additional receptors recognize host-derived signals, called damage-associated molecular patterns (DAMPs), that are released as a result of perturbations of tissue homeostasis caused by microbial or non-microbial insults, allowing a general sensing of stressed tissue[3].

Inflammasomes are a group of protein complexes that recognize a diverse set of inflammation-inducing stimuli that include PAMPs and DAMPs and that control the production of important pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-18 (refs 4, 5). Furthermore, they have been found to regulate other important aspects of inflammation and tissue repair such as pyroptosis, a form of cell death. Recent studies have characterized distinct molecular activation mechanisms for several sensor proteins and have identified a multitude of ligands of both endogenous and exogenous origins. Moreover, the diverse functions of these complexes in antimicrobial responses, as well as in multifaceted diseases such as metabolic syndrome and inflammatory bowel disease (IBD), have started to be revealed. Importantly, mutations in components of inflammasome complexes have been associated with a propensity for the development of several immune-mediated diseases in humans. We review several of these conditions, discuss the different models that have been proposed for inflammasome involvement in normal and aberrant immune response, and highlight the challenges and future directions for this field.

Inflammasomes are intracellular innate immune sensors

Inflammasomes control the activity of caspase-1

IL-1β is one of the quintessential pro-inflammatory cytokines that broadly affects inflammatory processes[6]. Tight control of its production is therefore required at the transcriptional and post-translational levels. IL-1β is synthesized as a pro-protein without a typical signal sequence that would allow its secretion, and instead its activation and cellular release are controlled by the cysteine protease caspase-1 (ref. 7). Similarly, caspase-1 is responsible for the processing and secretion of IL-18, as well as the secretion of other proteins such as IL-1α and fibroblast growth factor-2 through an unconventional protein secretion pathway[8]. Moreover, caspase-1 is required for pyroptosis, a form of cell death frequently observed during microbial infections that combines characteristics of apoptosis (DNA fragmentation) and necrosis (inflammation and cytokine release)[9]. Like other caspases, caspase-1 is synthesized as an inactive zymogen (pro-caspase-1) and becomes proteolytically active only after controlled dimerization in inflammasomes that are built around one of several different molecules[4,5,10–18] (Fig. 1). Whereas the leucine-rich repeat (LRR) domain is thought to be involved in autoinhibition that is disabled on direct or indirect sensing of the activating signal, the nucleotide-binding domain (NBD) is involved in the regulation of homo-oligomerization or hetero-oligomerization, which is required for inflammasome assembly. On receiving an activating signal, inflammasome sensors recruit pro-caspase-1 (which has a caspase activation and recruitment domain (CARD)) either directly through homotypic binding of CARD or indirectly through a pyrin domain (PYD) by means of the adaptor apoptosis-associated speck-like protein containing a CARD (ASC), which contains a PYD and a CARD.

Diverse signals induce inflammasome formation

Inflammasome assembly is unique in its induction by a variety of both exogenous and endogenous signals. The range of activation signals sensed by each protein is distinct, but may include overlapping signals (Box 1). Whereas the AIM2 and NLRC4 inflammasomes are activated only by specific PAMPs, double-stranded DNA (dsDNA) and specific bacterial proteins, respectively[15–17], NLRP3 is activated by a large variety of signals, including PAMPs, DAMPs and bacterial toxins[19–22]. The structural diversity of ligands activating the NLRP3 inflammasome is in striking contrast to other innate pattern recognition receptors such as Toll-like receptors, which usually recognize more confined structural motifs[23]. Several models have therefore been proposed to explain how all of these signals could activate the NLRP3 inflammasome (Box 1). These non-exclusive mechanisms include both direct and indirect signal recognition mediated by additional accessory proteins[21,24,25]. We discuss the nature of some of these NLRP3-activating signals in the context of the pathophysiology of several diseases, but they have been more extensively reviewed elsewhere[4,5].

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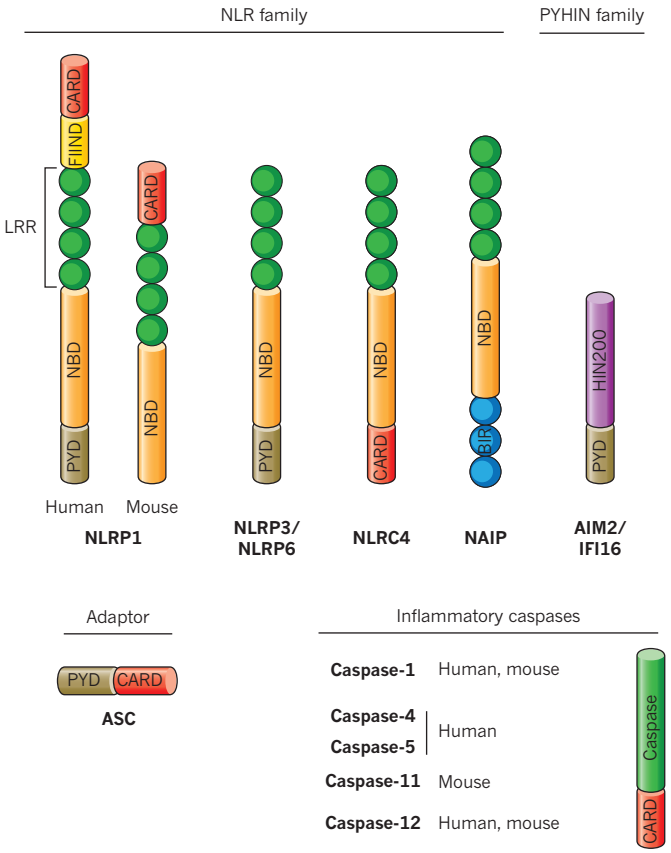


Figure 1 | Domain organization of inflammasome proteins. The identified core components belong to two families, the NOD-like receptor (NLR) family and the PYHIN (pyrin and HIN200 (haematopoietic interferon-inducible nuclear antigens with 200 amino-acid repeats) domain-containing protein) family. The NLR family members include NLRP1, NLRP2 (not shown), NLRP3, NLRP6, NLRC4 and, potentially, NLRP12. They all contain a nucleotide-binding domain (NBD), carboxy-terminal leucine-rich repeat (LRR), and can contain either a PYD or a caspase activation and recruitment domain (CARD) or both. The PYHIN family members AIM2 and IFI16 are characterized by having, in addition to a PYD, a HIN200 domain, which is involved in ligand binding. BIR, baculoviral inhibition of apoptosis repeat domain; FIIND, domain with function to find.

Regulation of inflammasome activity

Inflammasome activity needs to be tightly regulated by the host to avoid the excess production of cytokines or overt cell death. Regulation occurs at transcriptional and post-transcriptional levels. First, the expression of inflammasome sensors, in particular NLRP3, is relatively low in many cell types and requires a priming signal to be induced[26]. This signal is frequently referred to as signal I and can be provided by microbial ligands, cytokines or reactive oxygen species (ROS). In addition, alternative splicing of inflammasome components generates protein variants with different activities. Indeed, splice variants of ASC have been identified with distinct abilities to serve as an inflammasome adaptor, with one variant even having inhibitory activity[27]. Furthermore, the host expresses proteins that regulate inflammasome activity mainly by sequestering inflammasome components through homotypic interaction of CARDs or PYDs or through directly inhibiting caspase-1 function[28,29]. Similarly, subcellular location and trafficking of inflammasome components seems to be important for the regulation of inflammasome activity because ASC is found mainly in the nucleus in resting cells[30]. Another level of regulation may occur through the assembly of complexes with different components, suggested by the observation that cytokine processing downstream of NLRC4 activation is ASC dependent, whereas pyroptosis is independent of ASC[31]. In addition, it has been suggested that differential processing of caspase-1 might contribute to this finding because catalytically active caspase-1 that has been rendered unable to perform autocleavage is impaired in its ability to cleave cytokines, but it is still able to initiate pyroptosis on NLRC4-mediated recognition of *Salmonella* infection[32]. Regulation of inflammasome activity is also achieved through crosstalk with cellular stress-associated processes such as autophagy. Induction of autophagy leads to the degradation of cellular substrates such as protein aggregates and organelles in autolysosomes for the recycling of metabolites. Strikingly, cells deficient in autophagy have a decreased threshold for inflammasome activation[33]. This has been suggested to be a result of the impaired clearance of defective mitochondria resulting in elevated levels of ROS, hinting at an involvement of NLRP3 as sensor[34,35]. Another aspect of the regulation of inflammasome activity is its downregulation either through secreted factors or cell–cell interactions. Examples of these signals are type I interferons or interactions between $CD4^+$ T cells and macrophages or dendritic cells, respectively, leading to transcriptional and post-transcriptional downregulation of inflammasome activity[36,37]. All of these process probably cooperate in the temporal and spatial organization of inflammasome-mediated processes. In summary, the host has evolved distinct mechanisms to regulate inflammasome activation to prevent the dire consequences of inflammasome overactivation.

Regulation of inflammation by inflammasomes

Inflammasome-regulated processes depend on the simultaneous expression of the multiple inflammasome protein components in the same cell type of inflamed tissues. ASC and caspase-1 are found in many tissues and cell types, whereas the inflammasome sensors feature a more distinct expression pattern, suggesting tissue-specific mechanisms for sensing the microenvironment. In the following sections, we discuss how activation of inflammasomes in different lineages of cells regulates physiological reactions in the context of health and disease.

Inflammasomes and the antimicrobial response

In vivo, inflammasomes have been shown to participate in the antimicrobial innate immune response[38]. The most widely studied inflammasome in this regard is the NLRP3 inflammasome, shown to be involved in antibacterial, viral, fungal and parasitic immune responses. Despite the evidence linking the NLRP3 inflammasome to the immune response to infection, only in a minority of cases has inflammasome activation by direct recognition of the pathogen been documented; many studies have indicated inflammasome activation through induction of signals related to cellular stress and damage[4] (see Box 1). The influenza A virus is an example of an indirect viral NLRP3 inflammasome activator. On infection, recognition of viral RNA by means of Toll-like receptor 7 (TLR7) induces transcription of the NLRP3 inflammasome components[39]. Subsequently, the activity of the viral ion-channel protein M2 induces pH neutralization of the *trans*-Golgi network, leading to potassium efflux and ROS formation, which in turn induce NLRP3 inflammasome assembly. Recently, messenger RNA of microbial origin was shown to activate the NLRP3 inflammasome in a TRIF (TIR-domain-containing adaptor-inducing interferon-β)-dependent manner, providing a mechanism for NLRP3 recognition of infection and the resultant differentiation by the host between viable and non-viable bacteria, leading to the induction of a potent immune response only on exposure to live microbes[40]. Mouse NLRP1 inflammasome activation is central to the initiation of the antimicrobial response to *Bacillus anthracis* infection through caspase-1-induced pyroptosis of infected macrophages, which permits self-limitation of infection and initiation of an antimicrobial neutrophilic reaction[41]. Human NLRP1 forms an ASC-dependent inflammasome, whereas mouse NLRP1b may activate caspase-1 in an ASC-independent manner[42]. NLRP1, like NOD2 and NLRP3, can sense muramyl dipeptide (MDP), a building block of the bacterial cell wall, suggesting possible cooperative roles of the different pattern recognition receptors in the sensing of and

response to bacteria[43]. NLRC4 inflammasome activation is driven by type III and type IV secretion systems (T3SS and T4SS) of bacteria such as *Salmonella*, *Pseudomonas*, *Legionella* and *Yersinia*, which allow the cytoplasmic entry of the NLRC4 ligand flagellin, leading to activation of the NLRC4 pathway[31,44]. In addition, flagellin-independent activation of the NLRC4 inflammasome involves recognition of the T3SS rod protein PrgJ[45]. Interestingly, NLRC4 activation during *Legionella* infection is dependent on another NLR protein, NAIP5, but only partial dependence on NAIP5 was demonstrated for NLRC4 activation during *Pseudomonas* and *Salmonella* infection[45–47]. This apparent discrepancy was recently resolved by demonstrating that oligomerization of different NAIP family proteins with NLRC4 confers ligand specificity, so that a NAIP2–NLRC4 complex binds PrgJ and other structurally related rod proteins, whereas a NAIP5–NLRC4 complex binds flagellin[48]. Different pathogen receptors therefore assist NLRC4 in the recognition of microbial ligands, broadening the diversity of sensed structures. The recently discovered protein AIM2 recognizes bacterial and viral dsDNA, resulting in an antimicrobial response to intracellular pathogens, such as *Francisella tularensis*, *Listeria monocytogenes* and some DNA viruses[49,50].

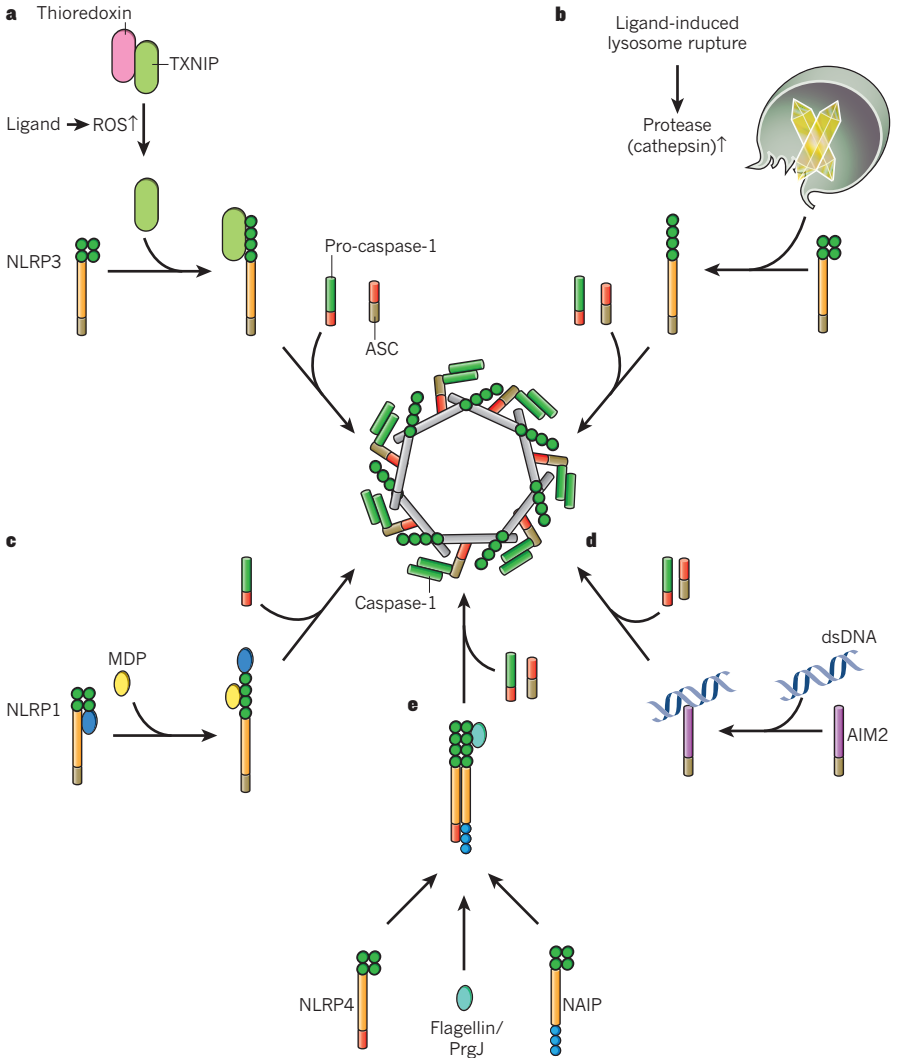
During microbial infection, distinct effector mechanisms of inflammasomes seem to be important for host defence. Although inflammasome-induced IL-1β and IL-18 are essential for the clearance of influenza virus and *Shigella*, respectively, pyroptosis has been suggested to be essential for caspase-1-mediated effects *in vivo* during infection by *Salmonella enterica* Typhimurium, *Legionella pneumophila* or *Burkholderia thailandensis*[51]. Altogether, it seems that the cooperative activity of distinct inflammasomes is pivotal in the host's ability to raise a protective immune response. This cooperation is illustrated during infection by *S. Typhimurium*, in which deficiencies in either NLRP3 or NLRC4 *in vivo* do not lead to increased bacterial infection, whereas mice deficient in both NLRC4 and NLRP3 have a similarly increased susceptibility to infection to that of caspase-1-deficient mice[52] (Fig. 2).

To avoid inducing inflammasome activation, many pathogens encode inhibitors of inflammasome function[53]. Frequently, these inhibitors, such as NS1 of influenza virus or Crm1 of cowpox virus, act on central inflammasome components and directly inhibit caspase-1 activity[54,55]. Other inhibitors, such as ORF63 of Kaposi's sarcoma-associated herpesvirus, bind NLR sensors and prevent the formation of NLRP1 and NLRP3 inflammasomes[56]. In general, the inhibition of inflammasome activity by the immune-evasive mechanisms of many microbes highlights the importance of these complexes in

BOX 1

Models for inflammasome activation

Inflammasomes are assembled after sensing a structurally diverse repertoire of PAMPs and DAMPs. Several models have been proposed to explain how these signals are sensed, including models based on recognition of general cellular stress (Fig. **a** and **b**) or on direct and indirect recognition of activation signals (Fig. **c**–**e**). NLRP3 senses the reactive oxygen species (ROS), which is produced in the cell (potentially by mitochondria) directly or indirectly by activators of the NLRP3 inflammasome. Increased amounts of ROS are sensed by a complex of thioredoxin and thioredoxin-interacting protein (TXNIP), leading to the dissociation of this complex. Subsequent binding of TXNIP to NLRP3 leads to the activation of NLRP3, the recruitment of ASC and pro-caspase-1, and formation of the active inflammasome complex (**a**). NLRP3 is activated after lysosome destabilization. The phagocytosis of specific crystalline and particulate structures can lead to lysosome destabilization and the release of lysosomal content, including proteases. These proteases could lead to proteolytic inactivation of a negative regulator or to proteolytic activation of a positive regulator of NLRP3, resulting in inflammasome assembly (**b**). NLRP1 and AIM2 sense the ligand directly. The direct binding of specific ligands (muramyl dipeptide (MDP) and double-stranded DNA (dsDNA)) can lead to conformational changes in NLRP1 and AIM2, resulting in inflammasome activation. Inflammasome formation in NLRP1 is independent of ASC (**c, d**). NAIP proteins sense bacterial proteins resulting in the recruitment of NLRC4 and assembly of the NLRC4 inflammasome (**e**).



antimicrobial immunity. Over the next few years, it will be important to further characterize how pathogens are sensed by inflammasomes and how inflammasomes interact with additional innate immune pathways to regulate antimicrobial immune responses *in vivo*.

Inflammasome activation by particulate compounds

In addition to infectious agents, there is evidence in mice and in humans that aberrant inflammasome activation by non-infectious agents may be linked to the pathogenesis of diseases characterized by sterile inflammation. For instance, crystal deposition diseases are a group of disorders in which inflammatory damage is elicited by exposure to exogenous or endogenous crystalline molecules. Examples of exogenous activators are silica and asbestos, whose endocytosis by pulmonary macrophages results in NLRP3 inflammasome activation involving ROS and lysosome destabilization, leading in turn to silicosis and asbestosis, respectively[24,57]. Similarly, aberrant formation of crystals from endogenous molecules such as monosodium urate (MSU) and calcium phosphate may lead to NLRP3 inflammasome activation in macrophages. Indeed, particular accumulation of MSU had long been noted during gout, but only recently has it been demonstrated that NLRP3-deficient mice feature defective MSU-induced neutrophil infiltration and inflammation[22] (Fig. 2). On the basis of these findings, clinical trials have been started to block IL-1β in gout; promising early results suggest that aberrant inflammasome activation drives inflammation in human crystal deposition disease. Furthermore, in patients with osteoarthritis, a common degenerative articular disorder, uric acid levels in synovial fluids are positively correlated with both IL-1β and IL-18 levels, as well as disease severity[58]. Calcium phosphate crystals were recently shown to activate NLRP3 (ref. 59). Hydroxyapatite crystals, a component of bone, are frequently found in osteoarthritis synovial fluid, activate IL-1β production by means of the NLRP3 inflammasome, and mediate inflammation and joint disease[59]. Future studies and clinical trials will be needed to explore whether targeting inflammasomes and their substrates are effective treatment options for these diseases.

An interesting example of inflammation that can be induced by an exogenous crystalline compound is the activation of the NLRP3 inflammasome by alum, an aluminium salt and the most commonly used adjuvant in human vaccines. Exposure of macrophages to alum *in vitro* leads to the NLRP3-dependent activation of caspase-1, and several studies have demonstrated that NLRP3 inflammasome-deficient mice have defects in alum-induced adaptive immune responses *in vivo*[60–62]. Several other studies have debated the role of the NLRP3 inflammasome in the adjuvant effect of alum, some suggesting that vaccination-induced adaptive immune responses may be ASC dependent but independent of caspase-1 (and hence independent of inflammasomes)[63–66]. A recent study suggested an alternative NLRP3-independent mechanism for alum's adjuvant effects, namely attachment to the plasma membrane of dendritic cells, leading to abortive phagocytosis and imprinting dendritic cells to induce a humoral immune response[67]. The precise mechanisms related to vaccination adjuvant effects and the possible contribution by inflammasomes remain to be clarified in future studies.

Inflammasomes and metabolic syndrome

The incidence of obesity worldwide has increased markedly in recent decades. Obesity is associated with multiorgan (namely pancreatic, adipose, hepatic, cardiac and muscle tissue) chronic metabolic and inflammatory alterations that together are termed 'metabolic syndrome'. These alterations include impaired insulin sensitivity, pancreatic β-cell dysfunction, non-alcoholic fatty liver disease and atherosclerosis. Unequivocal experimental and clinical evidence causally link IL-1β and IL-18 to the development of these metabolic pathologies and their complications[68,69]. However, the underlying molecular mechanisms that result in tissue-specific inflammasome activation in the context of obesity have only just begun to be explained.

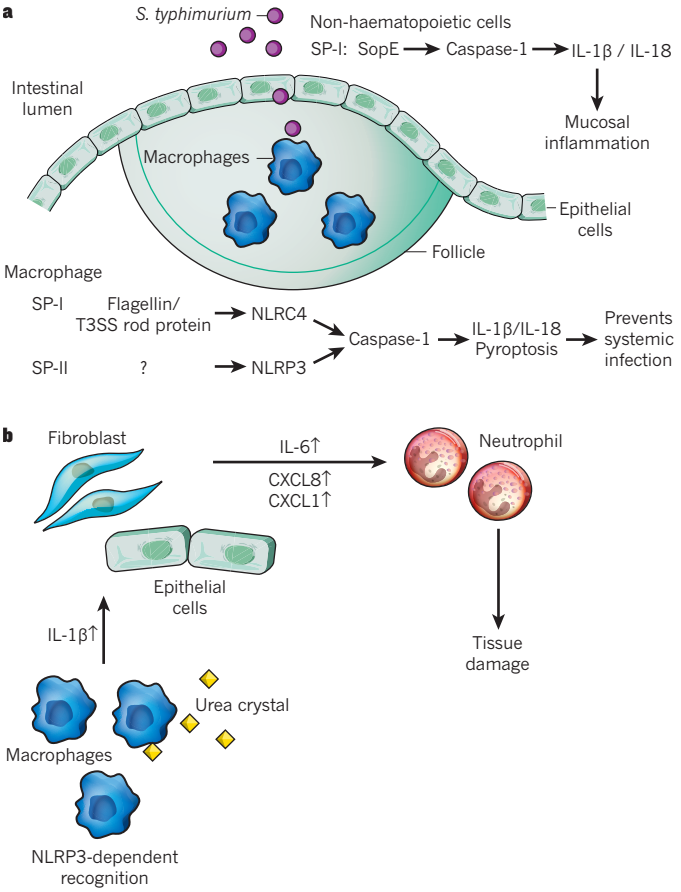


Figure 2 | Inflammasome activity regulates inflammation during microbial infection and autoinflammatory diseases. **a**, *S. typhimurium* enters the host by crossing through the intestinal epithelial barrier. M cells, a specialized epithelial cell type layering Peyer's patches, are particularly involved in the transcytosis of *Salmonella* and the infection of macrophages in Peyer's patches. The inflammasome and caspase-1 are involved in several cell types and at several steps of the infection. Injection of the bacterial effector protein SopE into epithelial cells induces the activation of caspase-1 independently of NLRP3 and NLRC4 through a process involving the GTPase Rac1. The resultant mucosal inflammation is dependent on IL-1β and IL-18 produced by non-haematopoietic cells. On infection of macrophages, the bacterial proteins flagellin and PrgJ (part of the T3SS) are sensed by means of NLRC4. This results in the activation of caspase-1, leading to both IL-1β/IL-18 processing and pyroptosis, which limit systemic infection. NLRP3 contributes to these processes by recognition of an unknown signal. **b**, Phagocytosis of monosodium urate (MSU) crystals by macrophages induces the NLRP3-dependent caspase-1 activation and release of IL-1β, which stimulates non-haematopoietic cells to produce IL-6 and chemokines (CXCL1 and CXCL8), attracting neutrophils. Activated neutrophils then cause tissue damage. Therapeutic blockade of IL-1β in humans ameliorates inflammatory bouts in gout.

Inflammasome activation regulates insulin signalling

NLRP3 inflammasome components and caspase-1 activation are increased in the adipose tissue and liver of obese mice and humans; moreover, their level of expression is directly correlated with the severity of type 2 diabetes mellitus (T2DM) in obese individuals[70]. During nutritional surplus, in addition to adipocyte hypertrophy owing to increased lipid storage, adipose tissue is infiltrated by classically activated, M1, macrophages that secrete pro-inflammatory cytokines[71]. NLRP3, ASC and caspase-1 are preferentially expressed in adipose-tissue-infiltrating macrophages, in which 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[70,72] (Fig. 3). Enhanced

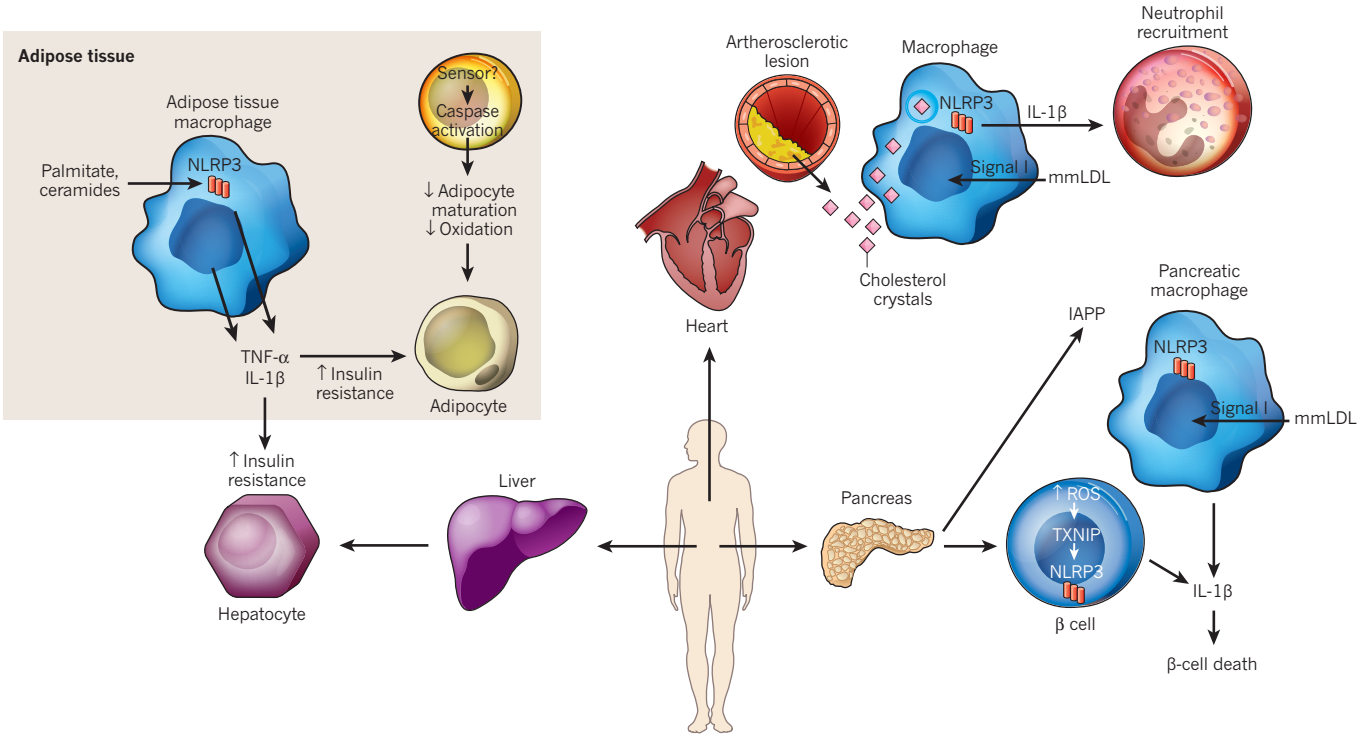


Figure 3 | The role of inflammasomes in metabolic syndrome. During obesity, the NLRP3 inflammasome is activated by obesity-associated DAMPs in multiple tissues and cell types; the resultant pro-inflammatory-induced state often leads to a deterioration in metabolic functions. In adipose tissue, palmitate and ceramides activate the NLRP3 inflammasome in infiltrating macrophages, which leads to an enhancement of insulin resistance. In addition, caspase-1 activation through an unknown sensor protein regulates adipocyte differentiation and fatty acid oxidation. In the pancreas, IAPP and increased mitochondrial ROS production activate the NLRP3 inflammasome in mmLDL-primed macrophages and β cells, respectively. The increased levels of IL-1β in pancreatic islets result in increased β-cell death and decreased insulin production. Minute cholesterol crystals in early atherosclerotic lesions activate the NLRP3 inflammasome in mmLDL-primed macrophages, promoting inflammatory-cell infiltration and increased atherosclerosis progression.

NLRP3-mediated caspase-1 activation in adipose-tissue-infiltrating macrophages of obese mice results in at least two deleterious consequences in insulin-sensitive tissues: first, IL-1β inhibits insulin signalling by direct serine phosphorylation of insulin receptor substrate 1 and induces the expression of tumour necrosis-factor-α, a well-characterized insulin-resistance-promoting cytokine; and second, IL-1β and IL-18 induce type 1 CD4 T helper cells in adipose tissue[70].

Inflammasomes also seem to have adipocyte-intrinsic functions. Caspase-1 is upregulated during adipocyte differentiation, in which it promotes insulin resistance through autocrine IL-1β-mediated effects; however, perhaps more interestingly, caspase-1-deficient precursors differentiate more efficiently into mature adipocytes and have an increased oxidation rate[73] (Fig. 3), which suggests that adipocyte-specific proteins may serve as caspase-1 substrates, leading to functional changes in adipose tissue of obese animals. Hence, *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ and *Casp1*$^{-/-}$ mice have been reported to have decreased weight gain and fat mass, as well as decreased insulin resistance. Interestingly, the loss of function of NLRP3 decreases but does not eliminate caspase-1 activation in adipose tissue or liver, suggesting that additional inflammasome sensors might contribute to the pathophysiology of obesity; furthermore, the nature of the stimuli that trigger caspase-1 activation during the accumulation of fat in adipocytes remains to be determined.

Inflammasome activation impairs β-cell function

Chronic hyperglycaemia as a result of peripheral insulin resistance is compensated for by increased insulin output by pancreatic β cells. Local inflammatory processes coupled with the toxic effects of glucose lead to accelerated mass loss of β cells and decreased insulin secretion over time, which prompts the progression from obesity and insulin resistance to overt T2DM. IL-1β, preferentially expressed by pancreatic infiltrating macrophages and to a smaller extent by β cells, has been implicated as a critical driver of β-cell death in conditions of chronic exposure to elevated concentrations of glucose[74]. Additional support for a pathological role of inflammasomes in T2DM comes from a recent report showing that glyburide, an insulin-secretion-promoting drug, suppresses NLRP3-mediated IL-1β release[75], and more importantly from human clinical trials of IL-1 receptor antagonist (IL-1RA) to treat T2DM that demonstrated improved glycaemic control and β-cell function[76].

In pancreatic islet failure during T2DM, NLRP3 inflammasomes may be activated by different mechanisms. First, hyperglycaemia triggers mitochondrial ROS production in β cells by increasing the activity of the electron transport chain, which induces thioredoxin-interacting protein (TXNIP) to dissociate from thioredoxin, leading to the activation of NLRP3 (ref. 25) (Fig. 3 and Box 1). Second, the severity of T2DM is closely correlated with the levels of deposition of amyloid polypeptide (IAPP, also known as amylin) in pancreatic islets[77]. Remarkably, IAPP has been shown to specifically activate the NLRP3 inflammasome in mouse pancreatic macrophages previously primed with the abundant minimally oxidized low-density lipoprotein (mmLDL) through a pathway that involves disruption of the phagolysosomal pathway[78] (Fig. 3). Further evidence for the role of IAPP and NLRP3 activation in pancreatic islet deterioration is provided by the observation that macrophages express more IL-1β in pancreatic islets of human *IAPP*-transgenic mice[78]. Overall, secreted IL-1β can then signal in an autocrine or paracrine manner to induce β-cell death or dysfunction and to promote the expression of chemotactic factors that further worsen immune-cell infiltration. It is important to mention that TXNIP is not required for NLRP3 activation in macrophages, strengthening the notion that the cellular milieu is a critical determinant of the way in which the NLRP3 inflammasome senses damage signals in the

context of metabolic abnormalities[78]. However, despite great advances in recent years, several questions remain unresolved, including the mechanisms of pro-IL-1β induction in β cells, the exact contribution of inflammasome activation in infiltrating macrophages and β cells to disease progression, and the potential roles of additional inflammasome sensors in pancreatic inflammatory processes.

Inflammasome activation and atherosclerosis

The inflammatory nature of atherosclerosis is well established, but the biological agents that trigger artery wall inflammation remain largely unknown. Cholesterol crystal deposition in arterial vessels has long been a pathognomonic feature of atherosclerosis; moreover, recent evidence suggests that they are present at early stages of atherosclerotic lesions, coinciding with the first appearance of inflammatory cells[79]. Similarly to IAPP, cholesterol crystals activate the NLRP3 inflammasome through phagolysosome destabilization in mmLDL-primed mouse and human macrophages[79] (Fig. 3). LDL-receptor-deficient mice (prone to atherosclerosis) reconstituted with bone marrow deficient in NLRP3, ASC or IL-1α/β are markedly resistant to the development of atherosclerosis, suggesting that NLRP3 inflammasome activation and IL-1 secretion from the haematopoietic compartment are key events in the early stages of disease[79]. Another model for atherosclerosis is the *Apoe*$^{-/-}$ mouse, which develops severe hypercholesterolaemia and spontaneous atherosclerosis when being fed with a high-fat diet[80]. Earlier reports have demonstrated that IL-1-receptor-deficient *Apoe*$^{-/-}$ mice and IL-1RA-treated *Apoe*$^{-/-}$ mice showed decreased atherosclerosis, suggesting a role for the inflammasome in atherogenesis in this mouse model[81,82]. Interestingly, using mice deficient in both ApoE and different components of the NLRP3 inflammasome, Menu *et al.*[83] showed that atherosclerosis progresses independently of the NLRP3 inflammasome in this context. The most reasonable explanations for the discrepancies between the two studies lie in the differences between atherosclerosis models (*Apoe*$^{-/-}$ versus *Ldlr*$^{-/-}$) and in a putative role for IL-1α in the *Apoe*$^{-/-}$ mouse model. However, it also raises the interesting possibility that environmental factors could account for the contradictory phenotypes. Clearly, further research is warranted regarding the implication of the inflammasomes in the pathogenesis of atherosclerosis and their role in non-haematopoietic cells during atherosclerotic plaque formation.

Inflammasomes and the mucosal immune response

In the gastrointestinal mucosa, the host is separated from an immense microbial ecosystem by only a single layer of epithelial cells[84]. To avoid ongoing inflammatory reactions to commensal microbes and food antigens, while preserving the ability to react to pathogenic insults, mammals have evolved a complex mucosal immune system composed of epithelial and stromal cells acting together with subsets of cells of haematopoietic origin. These cells interact closely with each other and with the surrounding microbial milieu. When such interactions are perturbed, autoinflammation may develop, potentially leading to IBD. The ability of inflammasomes to recognize exogenous and endogenous signals has led to several studies characterizing their role during chemical-induced intestinal autoinflammation, a model for human IBD. Interestingly, conflicting results have been reported by several groups (Fig. 4). Using a common acute and chronic epithelial injury colitis model based on the administration of dextran sulphate to mice, several groups reported decreased disease severity in mice deficient in caspase-1 or NLRP3, which correlated with lower IL-1β production during disease[85,86]. Using the same model, other groups found that mice deficient in NLRP3, ASC and caspase-1 show an exacerbated disease severity[87–90]. In these reports, a role was suggested for the NLRP3 inflammasome in the promotion of tissue regeneration in response to injury. Although variations in these results could be explained by differences in experimental designs, a recent paper[91] has offered an alternative explanation. This study reported that an NLRP6 inflammasome participates in the steady-state regulation of the commensal microflora. Deficiency in this inflammasome is associated with an alteration in the microflora communities and the emergence of normally suppressed bacteria that have pro-inflammatory activity. It was suggested that regulation of the microflora by epithelial inflammasomes is mediated, at least in part, through the induction of basal secretion of IL-18 by epithelial cells. Discrepancies in the earlier studies may therefore be explained by differences in microflora communities in different animal facilities, coupled with the inability of NLRP6-inflammasome-deficient mice (*Nlrp6*$^{-/-}$, *Asc*$^{-/-}$, *Casp1*$^{-/-}$) to regulate the microflora in a given facility.

Phenotypic alterations may be induced by differences in animal housing; wild-type mice housed with NLRP6-inflammasome-deficient mice acquire their colitogenic microflora and develop phenotypes that may be profoundly different from that of wild-type mice housed alone. The factors inducing the formation of the NLRP6 inflammasome and the precise effector mechanisms for regulation of the microflora remain to be studied. In addition to NLRP6, other inflammasomes that are expressed mainly within the haematopoietic compartment, such as the NLRP3 inflammasome, may also function by regulating the microflora, as well as the autoinflammatory process itself. In the mucosal environment, inflammasomes therefore function in the sensing of pathogens and the commensal flora by non-haematopoietic cells, such as the epithelial layers, but also by haematopoietic cells. They cooperate to maintain tolerance towards commensal microbes and to initiate a potent immune response towards pathogens (Fig. 4). Distinct inflammasomes expressed in different cell lineages may orchestrate these seemingly opposite functions during acute mucosal inflammation.

It has been suggested that inflammasomes participate in inflammation-induced tumorigenesis, a common complication of chronic intestinal autoinflammation (such as IBD), yet mechanisms accounting for this regulatory effect remain elusive[87,89]. Two recent reports suggest that NLRP6 deficiency may be associated with enhanced inflammation-induced tumorigenesis through impaired mucosal self-renewal and proliferation mediated by alterations in the intestinal stem-cell niche, or alternatively by an impaired haematopoietic immune response[92,93]. It remains to be determined whether the effect of NLRP6 on tumorigenesis is a direct consequence of NLRP6 deficiency in one or more intestinal cell types or whether it is indirectly induced by the documented alterations in the regulation of microflora composition of these mice.

Alternatively, another report suggests that epithelial NLRC4 inflammasome activity may also participate in the prevention of intestinal inflammation-induced tumorigenesis, because NLRC4-deficient mice were more prone to the development of colonic neoplasms after the induction of chronic inflammation[94]. This effect was independent of inflammation or the composition of the intestinal microflora and was suggested to involve enhanced proliferation of epithelial cells and impaired apoptosis of colonic epithelial cells. The precise molecular mechanism linking the NLRC4 inflammasome dysfunction to increased tumorigenesis remains to be explored.

Future perspectives

The discovery of inflammasomes as protein platforms that control the processing of IL-1β and IL-18 constitutes a milestone in the innate immunology field. However, many questions remain unanswered, including those regarding the biochemical and genetic regulation of these protein complexes, as well as their roles in complex diseases. These, in our opinion, may be the focus of inflammasome research in coming years.

The mechanism through which sensor proteins recognize activating signals remains a critical unresolved issue in the field. Although *in vitro* reconstitution assays with purified components have been performed with NLRP1 and AIM2 and have shown direct binding of the respective ligands to both sensors, intense research has not been successful in identifying such direct interactions with any of the other inflammasome sensors[15–17,43]. Hence, it is plausible that as yet unidentified proteins may facilitate the recognition of structurally diverse activating

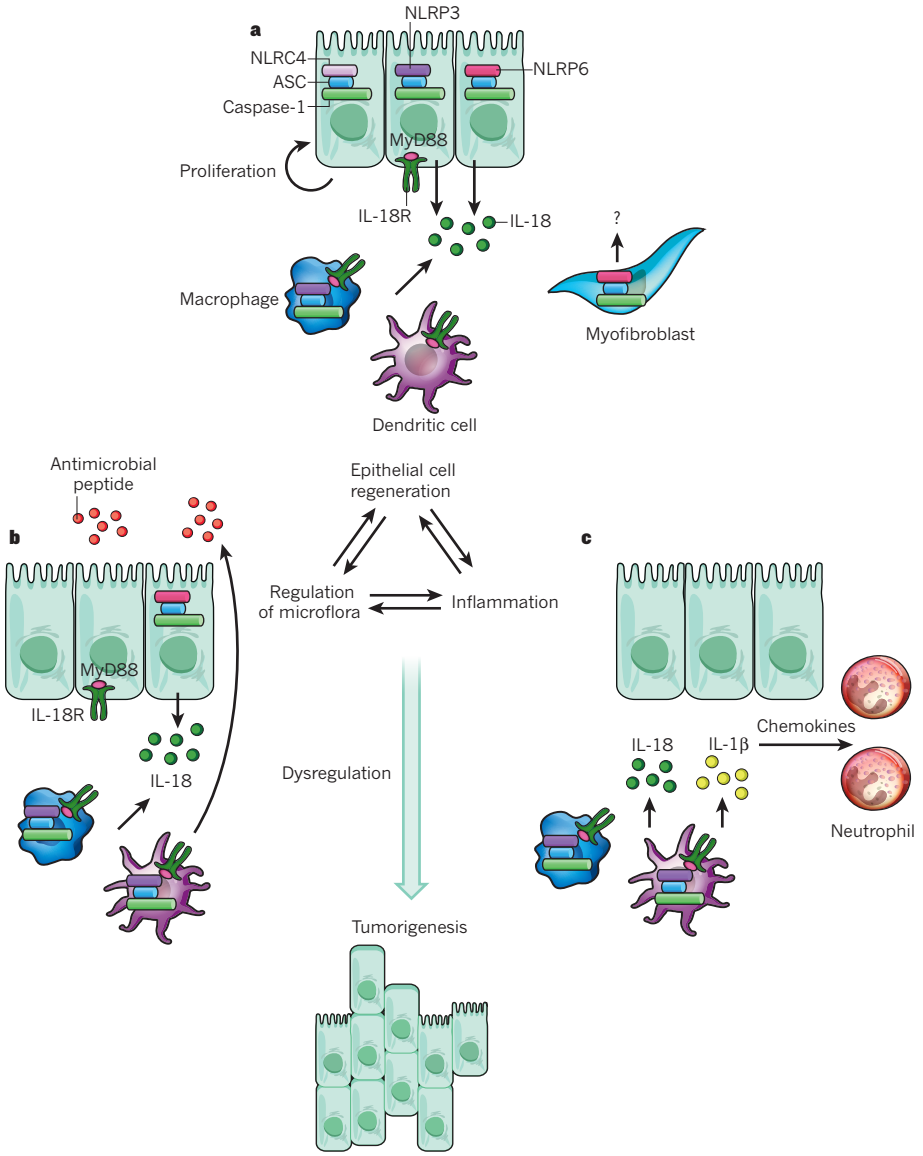


Figure 4 | Inflammasomes regulate multiple aspects of tissue homeostasis and immune response in the mucosa. NLRP3, NLRP6 and NLRC4 inflammasomes are formed in many lineages of haematopoietic and non-haematopoietic cells present in the intestine. The activity of these inflammasomes regulates epithelial cell regeneration, the microflora and mucosal inflammation. All of these processes are interconnected, and the dysregulation of any of them may increase the risk of tumorigenesis. **a**, Epithelial cell regeneration: recognition of as yet unknown signals leads to the processing of pro-IL-18 and potentially unidentified effectors through NLRP3, NLRP6 and NLRC4. This allows the regulation of epithelial regeneration on injury, through effects on epithelial cells, lamina propria immune cells, myofibroblasts and intestinal epithelial stem cells. **b**, Regulation of microflora: during the steady state, the production of IL-18 through NLRP3 and NLRP6 in the colon participates in the regulation of a 'healthy' microflora, potentially through regulation of antimicrobial peptide secretion by epithelial cells and immune cell subsets. Disruption of the NLRP6 inflammasome results in aberrant regulation of the intestinal microflora, leading to the emergence of pro-colitogenic bacteria. **c**, Inflammation: after breaching of the intestinal epithelial barrier, the NLRP3 inflammasome is involved in the regulation of intestinal inflammation in response to microbial invasion. Both IL-1β and IL-18 are suggested to act as effector molecules, leading to the recruitment of multiple cell subsets, including neutrophils, that promote local inflammation.

signals and ligands by NLRP3. Such 'bridging' mechanisms have been suggested to link cellular stress (namely potassium efflux, generation of ROS and lysosome destabilization) and microbial proteins to NLRP3 (refs 21, 24, 25). However, the exact identity of these bridging molecules remains to be fully characterized. Indeed, for the NLRC4 inflammasomes, NAIP proteins were identified as additional components that are responsible for the specificity of microbial ligands recognized by the complexes[48]. Another open issue is the interesting observation that several phenotypes related to a deficiency of common inflammasome effectors and adaptors cannot be attributed to the well-known NLR and PYHIN molecular sensors and their ligands, indicating the existence of additional sensors and ligands to be identified. An example of a recently identified sensor is IFI16, a sensor of viral DNA[18].

The early discovery that mutations in inflammasome components in humans are the cause of several inherited autoinflammatory diseases that can be treated by neutralizing IL-1β illustrates the potential detrimental effects of overactivation of inflammasomes[95]. However, recent evidence from mouse studies on the role of inflammasomes in mucosal immunology indicates a compartmentalized and dual function (detrimental versus beneficial). To dissect the contributions of inflammasomes in different cell types and tissues, novel tools such as conditional gene-knockout mice will have to be developed. This would allow better investigation of areas that bear fundamental importance and present complex challenges to the research community, such as the involvement of inflammasome signalling in circuits regulating metabolism, inflammation and the intestinal microflora in mice and humans[96].

It is worth noting that NLRP6 inflammasome deficiency was recently shown to associate with expansion of an autoinflammation-promoting microbiota including bacterial taxa such as Prevotellaceae and TM7 (ref. 91). The mechanisms by which inflammasomes sense microflora members or communities, regulate tissue repair and regeneration, and orchestrate mucosal immune responses during the steady state and inflammation remain to be fully characterized. Interestingly, both inflammasome deficiency and alterations in the intestinal microflora were previously linked in separate studies to a propensity for the development of elements of the metabolic syndrome, such as obesity and atherosclerosis, in mice and humans[97,98]. Indeed, recent characterization of the microflora community in a cohort of individuals of European origin identified the bacterial family Prevotellaceae as a prominent component of one human microflora cluster that featured a trend towards increased body mass index[99]. Moreover, faecal contents of rural African children featured expanded representation of Prevotellaceae that was absent in their European counterparts, probably reflecting a combination of genetic and environmental factors that differentially regulate the microflora composition[100]. Whether the regulation of the gut flora by inflammasomes affects weight, metabolism and inflammation is expected to become a major interest of the field.

Whereas the first decade of inflammasome research has been characterized mostly by *in vitro* studies and *in vivo* studies with small animal models, the contribution of altered inflammasome function to complex human disease is expected to gradually take centre stage. This, together with a vast effort to discover and develop small-molecule approaches to the specific inhibition of inflammasome activation, may pave the way to therapeutic intervention targeting inflammasome-regulated pathways that are involved in the pathogenesis of human disease. ■

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