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AIM2 inflammasome in infection, cancer and autoimmunity: role in DNA sensing, inflammation and innate immunity

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Abstract

Recognition of DNA by the cell is an important immunological signature that marks the initiation of an innate immune response. AIM2 is a cytoplasmic sensor that recognizes dsDNA of microbial or host origin. Upon binding to DNA, AIM2 assembles a multi-protein complex called the inflammasome, which drives pyroptosis and proteolytic cleavage of the pro-inflammatory cytokines pro-IL-1β and pro-IL-18. Release of microbial DNA into the cytoplasm during infection by *Francisella*, *Listeria*, *Mycobacterium*, mouse cytomegalovirus, vaccinia virus, *Aspergillus* and *Plasmodium* species leads to activation of the AIM2 inflammasome. In contrast, inappropriate recognition of cytoplasmic self-DNA by AIM2 contributes to the development of psoriasis, dermatitis, arthritis and other autoimmune and inflammatory diseases. Inflammasome-independent functions of AIM2 have also been described, including the regulation of the intestinal stem cell proliferation and the gut microbiota ecology in the control of colorectal cancer. In this review we provide an overview of the latest research on AIM2 inflammasome and its role in infection, cancer and autoimmunity.

Keywords

AIM2 inflammasome; autoimmunity; cancer; DNA sensing; bacterial/viral infection; gut microbiota

Introduction

DNA recognition by innate immune receptors triggers a myriad of immunological responses that are both beneficial and detrimental to the host. The discovery of Toll-like receptor 9 (TLR9) as a membrane-associated sensor of bacterial CpG DNA provides evidence for the existence of host receptors that specifically mediate immune responses to DNA [1]. Translocation of microbial or mammalian DNA into the cytoplasm of host cells further induces transcription of genes encoding type I interferon (IFN) molecules and inflammation independently of TLR9 [2, 3]. Recent advances in the field have identified multiple cytoplasmic DNA sensors which are responsible for transcriptional activity, including cyclic-GMP-AMP synthase (cGAS), STING, DDX41, Ku70, LRRFIP1, DNA-dependent

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Conflict of interest

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activator of IRFs (DAI, also known as ZBP1) and IFI16 (reviewed elsewhere [4–6]). Of these DNA sensors, cGAS binds to double-stranded DNA (dsDNA), resulting in a conformational change in cGAS that allows it to convert ATP and GTP to a cyclic dinucleotide cyclic-GMP-AMP (cGAMP) [7]. cGAMP then binds and activates STING to induce transcription of genes encoding type I IFN and pro-inflammatory cytokines via the transcription factors IRF3 and NF-κB, respectively (reviewed in [7]). The molecular basis underlying recognition of DNA by the other aforementioned cytoplasmic DNA sensors is less understood.

DNA introduced into the cytoplasm also induces IL-1β secretion and pyroptosis [8, 9], and these responses are dependent on the activity of a cytoplasmic caspase-1-containing complex known as the inflammasome [10]. In 2009, four groups independently identified AIM2 as the sensor that triggers inflammasome activation, pyroptosis and release of IL-1β and IL-18 in response to intracellularly-delivered dsDNA [11–14].

AIM2 consists of a C-terminal HIN-200 domain, which binds directly to dsDNA, and an N-terminal pyrin domain (PYD), which interacts with the PYD of the bipartite PYD-CARD-containing inflammasome adaptor protein ASC (apoptosis-associated speck-like protein containing a carboxy-terminal CARD) [10]. The CARD of ASC binds the CARD of pro-caspase-1, forming a macromolecular complex fulfilling the basic structural elements of an inflammasome (Figure 1) [10]. AIM2, IFI16 and other pyrin and HIN domain-containing (PYHIN) proteins form the AIM2-like receptor family [15–17].

AIM2 recognizes dsDNA in a sequence-independent manner; however, the DNA sequence must be at least 80 base pairs in length [18]. Elucidation of the crystal structure of AIM2 provided insights into the activation mechanism of this DNA-sensing inflammasome. The PYD and HIN-200 domain of AIM2 form an intramolecular complex and are maintained in an autoinhibitory state during homeostasis (Figure 1) [18, 19]. Binding of dsDNA to the HIN-200 domain displaces PYD from the intramolecular complex, liberating PYD for interaction with ASC [18]. The sugar-phosphate backbone of dsDNA interacts with the positively-charged HIN-200 domain via electrostatic attraction, allowing sequence-independent recognition of DNA by AIM2. The PYD of AIM2 can also self-oligomerize to induce the activation of the AIM2 inflammasome [20, 21]. Activation of AIM2 or a related inflammasome sensor NLRP3 initiates polymerization of the PYD of ASC [22, 23], ultimately forming a single and visually distinct inflammasome speck that is readily observed in primary macrophages and DCs [24–29]. Recent work has even suggested that the role of PYD of AIM2 is not to maintain autoinhibition, but to oligomerize and drive filament formation [30].

Activation of the AIM2 inflammasome and other canonical inflammasomes results in a type of inflammatory cell death called pyroptosis [10], which is mediated, in part, by the inflammatory caspase substrate gasdermin D [31, 32] (Figure 1). Activation of the AIM2 inflammasome is tightly regulated by the cell and requires phosphorylation and linear ubiquitination of ASC [33, 34]. Autophagy can mediate degradation of the AIM2 inflammasome to terminate inflammatory responses [35]. Furthermore, several proteins produced in the cell have the ability to inhibit activation of the AIM2 inflammasome [14,

36–43]. These include the PYD-containing proteins POP1 [39] and POP3 [36] in human cells and the bipartite protein containing two HIN domains, p202, in mouse cells [14, 40–43].

Here, we summarize the latest research on the regulation of AIM2 inflammasome, and its role in pathogen recognition after infection, cancer and autoimmunity.

AIM2 in the response to infection

Bacterial infection

During infection of a host cell, microbial DNA and other microbe-associated molecular patterns (MAMPs) are released into the cytoplasm, where they are recognized by cytoplasmic DNA sensors, i.e. cGAS, STING or AIM2. AIM2 resides in the cytoplasm and has been shown to provide immunosurveillance to the pathogenic bacteria *Francisella tularensis* Live Vaccine Strain (LVS), *F. tularensis* subspecies *novicida* (*F. novicida*), *Listeria monocytogenes*, *Streptococcus pneumonia*, *Mycobacterium* species, *Porphyromonas gingivalis*, *Staphylococcus aureus*, *Brucella abortus* and *Chlamydia muridarum* (Table 1) [26, 44–61]. *F. novicida* and *F. tularensis* LVS are the only bacterial pathogens known to exclusively activate the AIM2 inflammasome in mouse macrophages and DCs, whereas other bacteria have been shown to activate more than one inflammasome receptor in mouse and human cells [44–46]. In the human THP-1 macrophage-like cell line, both AIM2 and NLRP3 contribute to the activation of the inflammasome in response to *F. novicida* and *F. tularensis* LVS [48]. Interestingly, caspase-8 is recruited to the AIM2 inflammasome to drive apoptosis in *Francisella*-infected cells in the absence of caspase-1 [47, 62], suggesting a complex interplay between members of the caspase family.

Activation of the AIM2 inflammasome by *F. novicida and F. tularensis* LVS requires the ability of the bacteria to escape the vacuole into the host cytoplasm, a process mediated by a range of bacterial virulence factors, including the transcriptional regulator MglA and proteins encoded by the *Francisella*-pathogenicity island [26–28, 63, 64]. The *Francisella*-pathogenicity island is a genomic region which contains a cluster of 16 to 19 genes encoding virulence factors of the bacterium [65]. Similarly, *L. monocytogenes* must escape the vacuole and undergo bacteriolysis in order to induce the activation of the AIM2 inflammasome [49–52, 66]. Type I IFN potentiates the activity of the AIM2 inflammasome during bacterial infection [26–28, 67, 68]. In response to *F. novicida* infection, the DNA sensors cGAS, IFI204 and STING cooperate to detect small amounts of DNA released by the bacteria to drive production of type I IFN in mouse macrophages [27, 46, 69]. Type I IFN is then released to the outside of the cell, where it binds to the type I IFN receptor (IFNR) in an autocrine manner, activating the IFN-stimulated gene factor 3 (ISGF3) [70]. ISGF3 is comprised of the transcription factors STAT1, STAT2 and IRF9, which drives the transcription of IFN-stimulated genes (ISGs) [70]. A study has now demonstrated that, during infection with *F. novicida*, signaling via type I IFN induces the expression of the transcription factor IRF1, where IRF1 further drives expression of IFN-inducible GTPases called guanylate-binding proteins (GBPs) [27]. Induction of both IRF1 and GBPs are necessary to fully engage the AIM2 inflammasome by *F. novicida* infection (Figure 2) [27, 28].

GBPs are clustered over two locations in the genome of mice. Genes encoding GBP1, GBP2, GBP3, GBP5 and GBP7 are located on chromosome 3, whereas genes encoding GBP4, GBP6, GBP8, GBP9, GBP10 and GBP11 are found on chromosome 5 [71]. Of these, GBP2 and GBP5 are recruited to cytoplasmic *F. novicida* bacteria to drive bacterial killing, exposing abundant amounts of bacterial DNA for detection by AIM2 [27, 28]. GBP2 and GBP5 function in a non-redundant manner, and reconstitution of either GBP in type I IFN receptor 1-deficient macrophages cannot rescue inflammasome activation, indicating that type I IFN signaling activates GBPs, possibly via expression other IFN inducible proteins [27, 28]. Overall, mice lacking AIM2, caspase-1, IRF1 or GBPs have been shown to secrete reduced levels of IL-18 in response to *F. novicida* infection and are all hypersusceptible to *F. novicida* infection compared with wild type mice [27, 28, 44–46]. In agreement with this observation, antibody-mediated neutralization of IL-1β and IL-18 in wild type mice increases susceptibility to *F. novicida* infection [64].

The precise mechanism of bacterial killing mediated by GBPs remains unknown. Recent studies have shown that the antimicrobial activity of the gp91 subunit of NADPH oxidase (also known as NOX2), and inducible nitric oxide synthase are not required for mediating activation of the AIM2 inflammasome [28, 72]. However, the pharmacological inhibition of reactive oxygen species (ROS) and mitochondrial ROS partially reduces caspase-1 activation, and therefore, the release of IL-1β driven by *F. novicida* infection [28, 72]. ROS inhibition impairs the expression of IL-1β and TNF, arguing that further evidence is required to convincingly link ROS-mediated bacterial killing and activation of the AIM2 inflammasome [73]. It also remains a mystery as to why DNA molecules that are released to activate cGAS, STING and IFI204 are unable to activate AIM2 at this stage. One possibility is that the concentration of DNA that is sufficient to activate cGAS, STING and IFI204 is lower than the concentration required to activate AIM2. AIM2 is constitutively expressed in the cell, but its expression is also induced by IFN [11, 37, 38]. However, transfection of dsDNA into the cytoplasm can directly activate AIM2 independently of IFN [27, 28, 73], ruling out a requirement for “priming” in activation of the AIM2 inflammasome.

Bacteria have evolved virulence determinants to prevent release of DNA and other bacterial ligands and avoid cytoplasmic detection and clearance by inflammasomes. *F. tularensis* subspecies *tularensis* SchuS4 have been shown to induce low levels of inflammasome activation and IL-1β secretion in primary mouse bone marrow-derived macrophages (BMDMs), possibly due to enhanced resistance to H_2O_2 to protect itself from bacteriolysis, or other virulence factors that confer evasion of the immune system [72]. The putative lipid II flippase, MviN, and RipA, a protein used for intracellular replication, of *F. tularensis* LVS are both required to dampen AIM2 inflammasome responses [74, 75]. Further studies have shown that *F. tularensis* LVS or *F. novicida* mutants lacking MviN, RipA, and several membrane-associated proteins or proteins involved in O-antigen or LPS biosynthesis are hypersusceptible to intracellular lysis and DNA release in macrophages, providing a rationale for why these mutants induce elevated activation of the AIM2 inflammasome [63]. Genes encoding the 5-formyltetrahydrofolate cycloligase within the folate metabolic pathway and pseudouridine synthase in *F. tularensis* LVS have also been demonstrated to influence the magnitude of AIM2 inflammasome activation [76]. A more recent study

identified a clustered, regularly interspaced, short palindromic repeats-CRISPR associated (CRISPR-Cas) system used by *F. novicida* to strengthen the integrity of its bacterial membrane, leading to reduced DNA release in the cytoplasm [77]. Another example is found in *Legionella pneumophila*, which encodes an effector protein SdhA, shown to prevent rupture of the *Legionella*-containing vacuole and thereby minimizing the amount of bacterial DNA released into the cytoplasm [78].

There is limited evidence so far to support the existence of mechanisms used by bacteria to directly inhibit or evade activation of the AIM2 inflammasome. However, *F. tularensis* LVS and the virulent SchuS4 strain do suppress TLR2-dependent responses to reduce the level of pro-IL-1β available for cleavage by the inflammasome [79]. Overall, the AIM2 inflammasome is an effective antimicrobial machinery against certain bacterial pathogens.

Viral infection

Inflammasome responses play an essential role in the host protection against viral infection [80, 81]. Genetic materials from DNA viruses which enter the cytoplasm can be detected by AIM2, for instance mouse cytomegalovirus (MCMV), vaccinia virus and human papillomaviruses (Table 2) [11, 44, 82]. MCMV and vaccinia viruses robustly induce inflammasome responses in mouse macrophages in an AIM2-dependent manner (Figure 2) [11, 27, 44]. Further, *Aim2*$^{-/-}$ mice infected with MCMV have an impaired ability to secrete IL-18, carry a higher viral titre, and have reduced levels of IFN-γproducing NK cells compared with infected wild type mice [44]. Human papillomaviruses have also been shown to drive IL-1β and IL-18 release in human keratinocytes in an AIM2-dependent manner [82].

To date, there is no strong evidence in the literature to indicate that DNA viruses other than MCMV and vaccinia virus activate the AIM2 inflammasome. A recent study suggests that in the human glomerular mesangial cell line infected with hepatitis B virus, siRNA-mediated silencing of the gene encoding AIM2 leads to a reduced expression of IL-1β, IL-18 and caspase-1 [83]. Whether AIM2 directly mediates the recognition of viral DNA derived from hepatitis B virus in immune or non-immune cells has not been established. Comparison of the expression of AIM2 in patients with acute and chronic hepatitis B revealed that those at the acute stage expressed higher levels of AIM2 in peripheral blood mononuclear cells [84]. A subsequent study reported that 89.4% of the liver tissues collected from individuals with chronic hepatitis B virus infection were positive for AIM2 expression by immunohistochemistry, compared with only 8.7 % of those with chronic hepatitis C infection [85]. Expression of the gene encoding AIM2 has been reported to be significantly higher in kidney tissues of patients with hepatitis B virus-associated glomerulonephritis compared with patients with chronic glomerulonephritis [83].

In all cases, it is probable that viral DNA binds directly to AIM2 to trigger inflammasome activation, but the precise molecular mechanism that leads to exposure of the viral DNA for sensing by AIM2 is not entirely clear. Unlike *F. novicida* infection, type I IFN signaling, IRF1 and GBPs are dispensable for the activation of the AIM2 inflammasome by either MCMV infection or transfected dsDNA [27]. However, AIM2 does not respond to all DNA viruses. For example, adenovirus and herpesviruses HSV-1 and MHV-68 activate the NLRP3 or putative IFI16 inflammasome, rather than the AIM2 inflammasome in mouse

bone marrow-derived macrophages or mouse thioglycollate-elicited macrophages [8, 44, 80, 81]. During HSV-1 infection of human macrophages, the capsid that encapsulates the viral DNA is degraded by the proteasome, which releases DNA for recognition by IFI16 in the cytoplasm [86], suggesting that the inability of AIM2 to sense viral DNA is probably not due to a lack of viral DNA release in the cytoplasm. It might be possible that certain DNA viruses can strategically inhibit the ability of AIM2 to interact with their DNA. Alternatively, the DNA of certain viruses, such as Hepatitis B virus and other Hepadnaviruses, could be transcribed into RNA templates, which may serve as activators for NLRP3 [87–89]. Indeed, a precedent exists for indirect sensing of DNA by the RNA sensor RIG-I [90, 91]. RNA polymerase III transcribes AT-rich DNA into dsRNA transcripts carrying an uncapped 5′-triphosphate moiety, which has been shown to activate RIG-I [90, 91]. Conversely, a study has reported a role for AIM2 in driving IL-1β secretion in response to the RNA viruses [92]. Silencing of genes encoding AIM2 and caspase-1 reduces proteolytic cleavage and release of IL-1β in human dermal fibroblasts infected with the RNA viruses Chikungunya virus or West Nile virus [92]. How AIM2 might sense RNA viruses is still unclear, and further characterization of the molecular mechanism involved in the activation of the AIM2 inflammasome by viruses is required.

Other pathogens

In addition to bacteria and viruses, AIM2 has been shown to mediate pathogen recognition of, and host defense to, the fungal pathogen *Aspergillus fumigatus* and the protozoan *Plasmodium berghei* (Table 3) [93, 94]. AIM2 and NLRP3 function in a redundant fashion to confer inflammasome activation against *A. fumigatus* infection in mouse bone-marrow derived DCs and mice [93] Furthermore, mice lacking both AIM2 and NLRP3, ASC or caspase-1, and infected with *A. fumigatus*, are more susceptible than infected wild type mice [93]. The requirement for dual sensing of pathogens by both AIM2 and NLRP3 has also been observed in mouse BMDMs stimulated with *Plasmodium berghei*-infected red blood cells or synthetic and natural hemozoins [94]. AIM2 also directly recognizes *A. fumigatus* genomic DNA introduced into the cytoplasm by a transfection agent [93] or *P. falciparum* genomic DNA transported into the cytoplasm by hemozoins [94]. It would be interesting to identify additional pathogens that can activate the AIM2 inflammasome.

AIM2 role in cancer biology

AIM2 has also been shown to suppress the development of cancer [95, 96]. The gene encoding AIM2 was originally isolated from human melanoma cells [97]. Reduced expression and frequent frameshift microsatellite instability of the *AIM2* have been observed in tumor tissues from patients with colorectal cancer [98–101]. Colorectal cancer patients whose tissues have reduced AIM2 expression have a poorer prognosis compared with those with a higher level of AIM2 expression [98]. Reduced expression of AIM2 has also been reported in prostate cancer [102], whereas increased expression has been detected in nasopharyngeal carcinoma tumors [103, 104], oral squamous cell carcinoma [105], and lung adenocarcinoma [106]. The differential expression of AIM2 in a range of tumor tissues suggests that it may have unique roles in different types of cancer.

The mechanism for AIM2 in the regulation of tumorigenesis has been described in a mouse model of colitis-associated colorectal cancer [95, 96]. Two groups have recently demonstrated that AIM2 operates independently of the inflammasome to prevent colorectal cancer [95, 96]. Both studies found that *Aim2*$^{-/-}$ mice developed severe colitis, polyps and higher tumor burden upon administration of AOM and DSS [95, 96]. Although differential production of the major inflammatory mediators, including TNF and IL-6, was not observed between wild type and *Aim2*$^{-/-}$ mice, proliferation of enterocytes was more pronounced in *Aim2*$^{-/-}$ mice [95, 96]. Indeed, murine fibroblasts and colon cancer cell lines expressing an AIM2-encoding construct have been shown to have an impaired ability to undergo proliferation [40, 107]. Overexpression of AIM2 in colon cancer cell lines also induces cell cycle arrest, and the transformed cells exhibit a delayed transition from the late S-phase to the G2/M phase [107], suggesting that AIM2 plays a proliferation-inhibitory role in these cancer cell lines.

Furthermore, in another mouse model of intestinal cancer, *Aim2*$^{-/-}$ mice carrying aberrant activating β-catenin mutations failed to prevent the expansion of cancer-associated stem cells in the small and large intestine [95]. Similarly, *Aim2*$^{-/-}$ mice harboring the heterozygous mutation in the adenomatous polyposis coli (*Apc*) gene developed more tumors than mice carrying only the mutant *Apc* gene [96]. Intestinal stem cells lacking AIM2 proliferated more than wild type intestinal stem cells in organoid culture, and this proliferation was associated with increased activation of the kinase AKT [95, 96]. Wilson and colleagues identified DNA-PK, a kinase that can phosphorylate and activate AKT [108, 109], as a binding partner of AIM2, whereby AIM2 suppresses the activation of DNA-PK and DNA-PK-dependent phosphorylation of AKT at the serine residue 473 [96]. Indeed, treatment of *Aim2*$^{-/-}$ mice with an AKT inhibitor reduced tumor burden in *Aim2*$^{-/-}$ mice with colitis [96].

Our laboratory performed further analysis and found that *Aim2*$^{-/-}$ mice harbored a microbial ecology different to that of wild type mice [95]. Reciprocal exchange of the microbiota between *Aim2*$^{-/-}$ and wild type mice by means of co-housing substantially reduced tumorigenesis in *Aim2*$^{-/-}$ mice and increased tumorigenesis in wild type mice [95]. Collectively, these studies provide insights into the function of AIM2 in colorectal cancer, and highlight that potential therapies that inhibit the AKT pathway can be further investigated for treatment of cancer associated with AIM2 mutations [40, 95, 96, 107].

AIM2 in inflammatory, autoimmune and other pathological conditions

Given that the host DNA is normally sequestered in the nucleus or mitochondria, the accumulation of host DNA in the cytosol, due to impaired degradation or clearance or excess uptake of extracellular DNA from dying neighboring cells, could induce inflammation. For instance, accumulated DNA can serve as an endogenous danger signal, and has been shown to trigger AIM2-dependent release of IL-1β in skin cells, contributing to the pathogenesis of psoriasis [38]. Scavenging of DNA by the antimicrobial cathelicidin peptide LL-37 produced by the inflamed skin of psoriasis patients prevents overt activation the AIM2 inflammasome and IL-1β release [38]. Increased AIM2 expression has been observed in patients with acute and chronic skin conditions, including psoriasis, atopic

dermatitis, venous ulcera, contact dermatitis and experimental wounds in humans [38, 110]. In addition, expression of the gene encoding AIM2 is elevated in immune cells of male patients with systemic lupus erythematosus (SLE) and both increases and decreases in AIM2 expression have been observed in female patients with SLE [111, 112]. Further, DNA methylation of the gene encoding AIM2 is reduced in patients with SLE compared with their healthy siblings [113], suggesting that differential expression or epigenetic changes could be linked to development of the disease.

Increased expression of AIM2 has been reported in patients with inflammatory bowel diseases and liver inflammation [114–116]. For example, elevated expression of AIM2 has been detected in ascitic fluid macrophages collected from cirrhotic patients, compared with PBMCs from the same patients, or with $CD14^+$ macrophages from peripheral blood mononuclear cells of healthy individuals [115]. Increased expression of AIM2 and NLRP3 and elevated activation of caspase-1 and maturation of IL-1β have been found in liver tissues of mice with steatohepatitis [116].

Moreover, there is evidence to suggest that AIM2 is involved in inflammation and cell death of the brain. Cell-free DNA fragments are more frequently detected in the cerebrospinal fluid (CSF) of patients with traumatic brain injury than in CSF from nontrauma patients [117]. When human embryonic cortical neurons, which express AIM2 inflammasome components and have been shown to be capable of IL-1β release and cell death upon AIM2 activation with poly(dA:dT) transfection [117], were exposed to the CSF of traumatic brain injury patients, they exhibited increased AIM2 expression and caspase-1 activation compared with embryonic cortical neurons which had been exposed to the CSF of non-trauma patients [117]. A further study has shown that mice lacking AIM2 are more protected than wild type mice to ischemic brain injury [118]. Upon induction of focal cerebral ischemia, less activation of microglial cells and recruitment of leukocytes were found in *Aim2*$^{-/-}$ mice compared with wild type mice [118].

The inability to degrade self-DNA also contributes to the pathogenesis of autoimmune polyarthritis. Mice lacking the lysosomal endonuclease DNAse II (*Dnase II*$^{-/-}$ mice) are embryonically lethal, owing to an impaired ability to degrade self-DNA by macrophages [119]. Genomic deletion of type I IFN receptor (IFNAR) rescued *Dnase II*$^{-/-}$ mice from embryonic lethality [120]; however, the mice lacking both IFNAR and DNAse II (*Dnase II*$^{-/-}$*Ifnar*$^{-/-}$ mice) would eventually develop polyarthritis [121]. Intriguingly, genomic deletion of AIM2 was shown to prevent inflammasome activation, inflammatory cytokine production, macrophage infiltration in the joint and the development of arthritis in *Dnase II*$^{-/-}$*Ifnar*$^{-/-}$ mice [122, 123]. Genomic deletion of STING was also shown to protect *Dnase II*$^{-/-}$*Ifnar*$^{-/-}$ mice from joint inflammation [122], indicating that multiple DNA sensors might contribute to the inappropriate DNA recognition driving clinical manifestation. Overall, aberrant activation of AIM2 from self-DNA is a key driver of inflammatory and autoimmune diseases.

Conclusions

A wide range of microbial pathogens is sensed by AIM2 in mammalian cells. Recognition of DNA from pathogens by AIM2 leads to protective inflammasome-mediated host responses. Recent studies have demonstrated that AIM2 inflammasome also plays important roles in non-microbial diseases, highlighting the multifaceted nature of AIM2 beyond immunity to infectious diseases. In colorectal cancer, AIM2 orchestrates inflammasome-independent functions by suppressing stem cell proliferation, and contributes to maintenance of a healthy gut microbiota. The role of AIM2 inflammasome in other types of cancer should be further explored. Moreover, inappropriate recognition of self-DNA by AIM2 triggers detrimental inflammatory responses, leading to superficial and systemic inflammation. Inhibiting AIM2 inflammasome activity using synthetic inhibitors, such as suppressive oligodeoxynucleotides [124], or harnessing the power of endogenous AIM2 inhibitors, such as pyrin-containing proteins [36, 39] or antimicrobial cathelicidin peptides [38], could be investigated for their potential to control unwanted inflammation. An additional line of research could focus on understanding the complementary relationship between AIM2 and a plethora of other DNA sensors in the context of different cell types, tissues and organs. A holistic understanding of the biology of AIM2 could lead to improved immunosurveillance in the fight against infectious diseases and cancer, while avoiding debilitating inflammatory and autoimmune diseases.

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Abbreviations

ASC	apoptosis-associated speck-like protein containing a CARD
GBP	guanylate-binding protein
IRF	interferon-regulatory factor
ISGF3	IFN-stimulated gene factor 3

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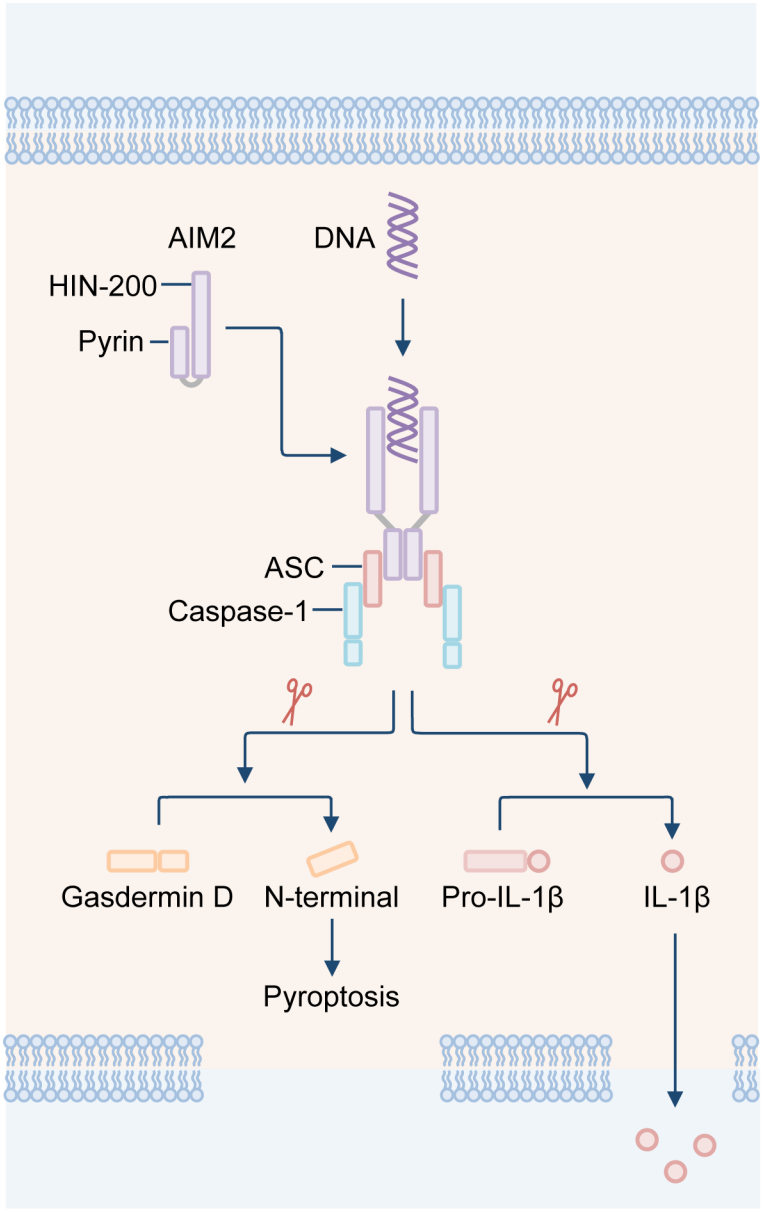

Figure 1. The molecular basis for the activation of the AIM2 inflammasome

The DNA sensor AIM2 is composed of an N-terminal pyrin domain and a C-terminal HIN-200 domain. The pyrin and HIN-200 domain of AIM2 form an intramolecular complex and are maintained in an autoinhibitory state. Cytoplasmic dsDNA induces activation of AIM2. The HIN-200 domain interacts with dsDNA in a sequence-independent manner, by binding to the sugar-phosphate backbone of dsDNA. The pyrin domain of AIM2 binds to the pyrin domain of ASC. CARD of ASC binds the CARD of pro-caspase-1, forming a macromolecular complex known as the AIM2 inflammasome. Activated caspase-1 drives cleavage of pro-IL-1β and pro-IL-18. Caspase-1 also cleaves the substrate gasdermin D. The N-terminal fragment of gasdermin D induces pyroptosis, allowing mature IL-1β and IL-18 to be released from the cell.

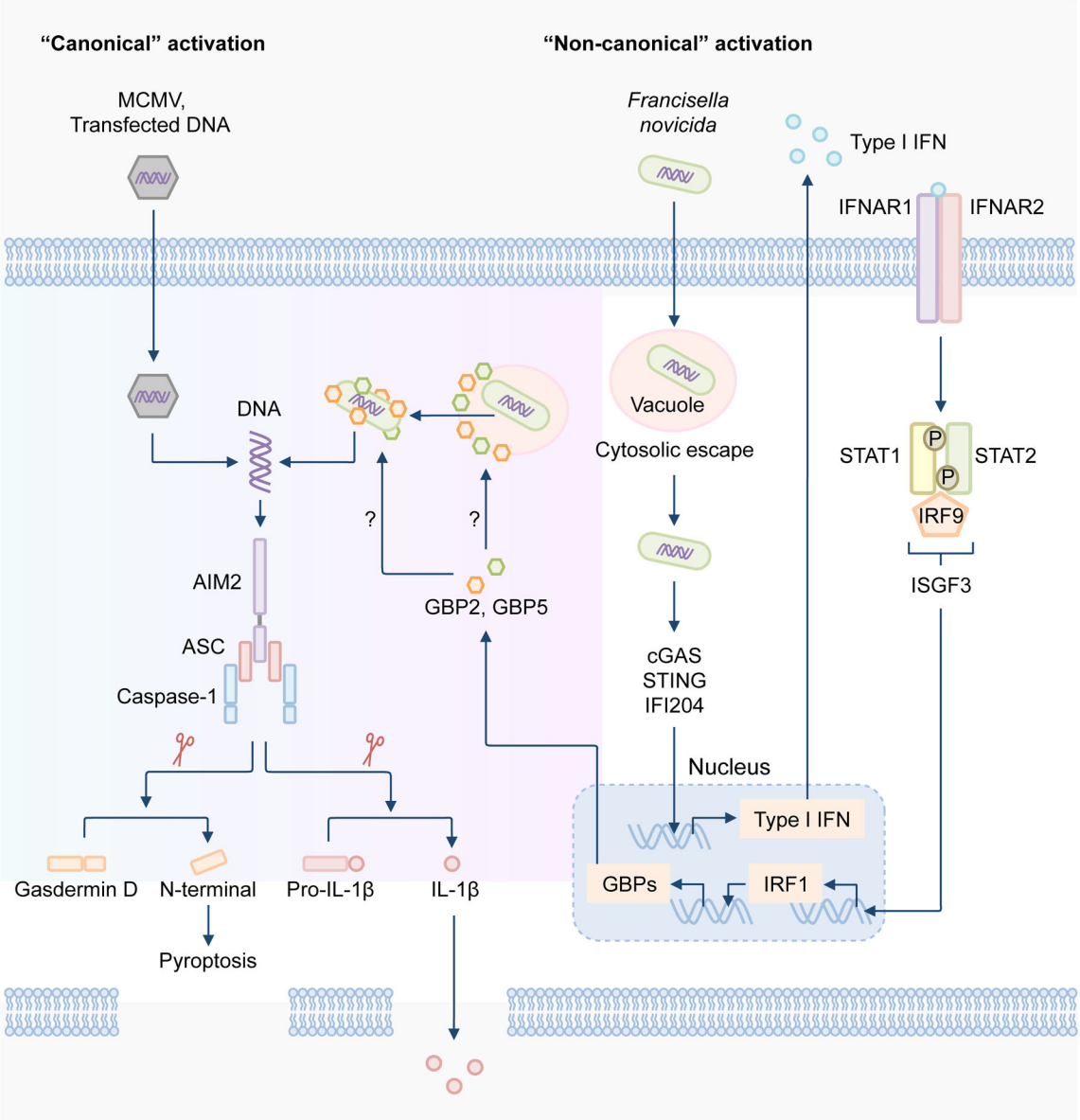

Figure 2. Regulation of the activation of the AIM2 inflammasome

The AIM2 inflammasome is activated by a number of microbial pathogens and dsDNA ligands, including the DNA virus mouse cytomegalovirus (MCMV), the cytosolic bacterium *Francisella novicida* and the dsDNA ligand poly(dA:dT). MCMV infection or transfection of poly(dA:dT) leads to "canonical" activation of the AIM2 inflammasome, which does not require the type I interferon (IFN) pathway. *F. novicida* infection activates the AIM2 inflammasome via a "non-canonical" pathway owing to its requirement for type I IFN, analogous to the non-canonical NLRP3 inflammasome pathway. Intracellular *F. novicida* releases DNA into the cytoplasm to activate the DNA sensors cGAS, STING and IFI204, which drive transcription of genes encoding type I IFN molecules. It remains unclear why the released DNA is unable to activate AIM2 at this stage, since AIM2 is constitutively expressed in the cell. Type I IFN provides a feedback loop to induce expression of the

transcription factor IRF1, which upregulates expression of the IFN-inducible GTPases, including GBP2 and GBP5. GBP2 and GBP5 are recruited to bacterial structures, however, whether they directly target the bacterial membrane or the membrane of intact *Francisella*-containing vacuole is unclear. Nevertheless, GBPs mediate bacterial killing, resulting in abundant release of bacterial DNA for recognition by AIM2. Assembly of the AIM2 inflammasome induces caspase-1-dependent cleavage of pro-IL-1β and pro-IL-18. Caspase-1 also drives cleavage of the substrate gasdermin D to induce pyroptosis.

Table 1

Bacteria recognized by the AIM2 inflammasome

Bacteria	Cells		Mice	
***Francisella tularensis* LVS or *F. tularensis* subspecies *novicida* (*F. novicida*)**	•	Reduced caspase-1 activation, IL-1β and IL-18 and pyroptosis in *Aim2*$^{-/-}$ mouse BMDMs or BMDCs [26, 44–47] • Reduced IL-1β in shRNA- silenced *Aim2* THP1 human monocytic cell line [48]	•	Reduced serum IL-18 levels 1d p.i. and increased bacterial burden 3d p.i. [44, 45]
Listeria monocytogenes	•	Reduced caspase-1 cleavage, IL-1β release and pyroptosis in *Aim2*-silenced mouse BMDMs [49–52]	•	N/A
Streptococcus pneumoniae	•	Reduced caspase-1 cleavage, IL-1β and IL-18 release and pyroptosis in *Aim2*-silenced peritoneal mouse macrophages [53]	•	N/A
Mycobacterium tuberculosis	•	Reduced IL-1β and IL-18 release in *Aim2*$^{-/-}$ and *Aim2*- silenced THP-1 human monocytic cell line [54, 55]	•	Increased overall, susceptibility, increased bacterial burden in the lungs and liver 4 weeks p.i. [56] • Reduced caspase-1 cleavage and increased infiltration of inflammatory cells in lungs. [56] • Reduced IL-1β in BALF and IL-18 in serum at 3 weeks p.i. [56] Reduced IFN-γ production by CD4^{+} T cells [56]
Mycobacterium bovis	•	Reduced caspase-1 cleavage, IL-1β release and pyroptosis in *Aim2*-silenced mouse macrophage J774A.1 [57]	•	N/A
Porphyromonas gingivalis	•	Reduced caspase-1 cleavage, IL-1β and pyroptosis in siRNA-silenced *Aim2* THP-1 human monocytic cell line [58]	•	N/A
Legionella pneumophila* Δ*SdhA	•	Reduced caspase-1 cleavage and IL-1β release in *Aim2*$^{-/-}$ mouse BMDMs [78]	•	N/A
Staphylococcus aureus	N/A		•	Increased susceptibility upon intracranial infection [59] • Reduced IL-1β, IL-6, CCL2 and CXCL10 in wound abscess [59]
Brucella abortus	•	Reduced caspase-1 cleavage, IL-1β and cell death in *Aim2*$^{-/-}$ mouse BMDMs [60]	•	Increased bacterial burden 4 weeks p.i [60]
Chlamydia muridarum	•	Reduced IL-1β and IL-18 in *Aim2*$^{-/-}$ mouse BMDMs [61]	•	N/A

Abbreviations: BALF, bronchoalveolar lavage fluid; BMDCs, bone marrow-derived dendritic cells; BMDMs, bone marrow-derived macrophages; p.i., post-infection. N/A, information not available.

Table 2

Viruses recognized by the AIM2 inflammasome

Viruses	Cells	Mice
Mouse cytomegalovirus (DNA virus)	• Reduced caspase-1 cleavage and IL-1β release *Aim2*$^{-/-}$ mouse peritoneal macrophages and BMDCs [44]	• Increased viral titers 36 h p.i. [44] • Reduced serum IL-18 level 36 h p.i. [44] • Reduced IFN-γ production [44]
***Vaccinia virus* (DNA virus)**	• Reduced caspase-1 cleavage and IL-1β release *Aim2*$^{-/-}$ mouse peritoneal macrophages and BMDCs [44]	• N/A
Human papillomaviruses (DNA virus)	• Reduced IL-1β and IL-18 release in *Aim2*-silenced human keratinocytes [82]	• N/A
Hepatitis B virus (DNA virus)	• Reduced gene expression of IL- 1β, IL-18 and caspase-1 in *Aim2*-silenced human glomerular mesangial cell line [83]	• N/A
Chikungunya virus (RNA virus)	• Reduced IL-1β release in *Aim2*- silenced human primary dermal fibroblasts [92]	• N/A
West Nile virus (RNA virus)	• Reduced IL-1β release in *Aim2*- silenced primary human dermal fibroblasts [92]	• N/A

Abbreviations: BMDCs, bone marrow-derived dendritic cells; p.i., post-infection. N/A, information not available.

Table 3

Fungi and parasites recognized by the AIM2 inflammasome

Fungi	Cells	Mice
Aspergillus fumigatus	Slight reduction in IL-1β and IL-18 in *Aim2*$^{-/-}$ mouse BMDCs owing to redundant roles with NLRP3 [93]	Not susceptible owing to redundant roles with NLRP3 [93]
Protozoa		
Plasmodium berghei	Slight reduction in IL-1β and pyroptosis in *Aim2*$^{-/-}$ mouse BMDMs infected with iRBCs, synthetic and natural hemozoin owing to redundant roles with NLRP3 [94]	Decreased neutrophils recruitment in peritoneal cavity owing to redundant roles with NLRP3, after 15 h p.i. [94]

Abbreviations: BMDCs, bone marrow-derived dendritic cells; iRBCs, infected red blood cells.