Common Differences: The Ability of Inflammasomes to Distinguish Between Self and Pathogen Nucleic acids during infection.

Christopher R. Lupfer[a,], Meagan D. Rippee-Brooks[a], and Paras K. Anand[b,*]*

[a] *Department of Biology, Missouri State University, Springfield, Missouri, USA*

[b] *Infectious Diseases and Immunity, Imperial College London, London W12 0NN, UK.*

***Correspondence should be addressed to:**

Paras K. Anand

Infectious Diseases and Immunity,

Department of Medicine, Imperial College London

The Commonwealth Building, Du Cane Road

London W12 0NN, UK

Tel: +44 (0) 20 8383 2063

E-mail: paras.anand@imperial.ac.uk

Or

Christopher R. Lupfer,

Department of Biology,

Missouri State University,

Springfield, Missouri, USA

E-mail: ChristopherLupfer@MissouriState.edu

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Abstract

The innate immune system detects the presence of pathogens based on detection of non-self. In other words, most pathogens possess intrinsic differences that can distinguish them from host cells. For example, bacteria and fungi have cell walls comprised of peptidoglycan and carbohydrates (like mannans), respectively. Germline encoded pattern recognition receptors (PRRs) of the Toll-like receptor (TLR) and C-type lectin receptor (CLR) family have the ability to detect such unique pathogen associated features. However, some TLRs and members of the RIG-I-like receptor (RLR), NOD-like receptor (NLR) or AIM2-like receptor (ALR) family can sense pathogen invasion based on pathogen nucleic acids. Nucleic acids are not unique to pathogens, thus raising the question of how such PRRs evolved to detect pathogens but not self. In this chapter, we will examine the PRRs that sense pathogen nucleic acids and subsequently activate the inflammasome signaling pathway. We will examine the selective mechanisms by which these receptors distinguish pathogens from ‘self’, and discuss the importance of such pathways in disease development in animal models and human patients.

Introduction

The immune system maintains and restores homeostasis of the host in response to physical injury, pathogen invasion, or metabolic dysfunction. The first line of defence is the innate immune system, which consists of leukocytes, epithelial cells and endothelial cells possessing the ability to recognize pathogens and damage to self-tissues. Through strategically placed cells present in almost every tissue in the host, the innate immune system responds almost immediately to disturbances in homeostasis by detecting the presence of pathogen associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs). PAMPs consist of conserved pathogen molecules including bacterial and fungal cell wall components or DNA and RNA from pathogens that are different from the host (CpG DNA from bacteria). In addition, DNA and RNA present in cellular or extracellular compartments where such molecules are not found under homeostasis (DNA in the cytoplasm) can further trigger an immune response (Bürckstümmer et al., 2009; Fernandes-Alnemri et al., 2009). DAMPs consist of cellular molecules released during cell damage and include ATP or nuclear proteins like HMGB1 released from damaged cells into the extracellular environment (Barlan et al., 2011b). Thus, the innate immune system can detect a wide range of pathogens or pathological conditions with a limited set of pattern recognition receptors (PRRs) that possess the ability to detect conserved PAMPs or DAMPs instead of responding to specific pathogen features individually (i.e. toxins or receptor proteins from pathogens).

Several classes of PRRs are involved in PAMP and DAMP recognition; however, this chapter will focus on a specific group of PRRs with the ability to recognize nucleic acids and activate an immune signaling complex known as the inflammasome. The

inflammasome is a multimeric protein structure consisting of an activating PRR, the adaptor protein ASC and proteases caspase-1 and caspase-11. Of note, not all inflammasomes require all caspases, thus the inclusion or exclusion of caspases provides some specificity to the immune response to different stimuli (Cunha et al., 2017; Pierini et al., 2012). The functions of the inflammasome are two-fold. Active caspase-1, and in some cases caspase-8, cleave cytokine precursors pro-IL-1β and pro-IL-18 into their active forms (Karki et al., 2015). Active caspase-1 and caspase-11 (Caspase-4 and-5 in humans) can also cleave the autoinhibitory C-terminal fragment of the protein gasdermin D, which then forms pores in the cell membrane resulting in an inflammatory form of cell death termed pyroptosis (He et al., 2015; Kayagaki et al., 2015; Shi et al., 2015). Although the overall function of the inflammasome is cytokine production and cell death, numerous PRRs can activate the inflammasome in response to a myriad of stimuli. Some members of the NOD-like receptor family of PRRs can activate the inflammasome. These include NLRP1, NLRP3, NLRP7, NLRP9b and NLRC4. AIM2 and IFI16 as well as the protein pyrin also activate the inflammasome. In addition, several NLR apoptosis inhibitory proteins (NAIP1, NAIP2, NAIP5, NAIP6) recognize ligands from bacterial secretion systems and activate NLRC4 (Kofoed and Vance, 2011; Rayamajhi et al., 2013; Yang et al., 2013a; Zhao et al., 2011). Of the PRRs listed above, NLRP3, AIM2, NLRP9b and IFI16 are reported to sense nucleic acids and activate the inflammasome. Of importance are the mechanisms by which these PRRs can distinguish between self and pathogen DNA or RNA, as there are very few structural differences between host and pathogen nucleic acids. The specificity of these PRRs for distinct pathogens or pathological conditions and their regulation are discussed in this chapter.

Overview of the AIM2 inflammasome

There are two pathways associated with the detection of cytoplasmic DNA: interferon-stimulatory DNA (ISD) pathway consisting of cGAS and STING and the caspase-1 inflammasome pathway (Gray et al., 2016). Here, the inflammasome pathway will be discussed in more detail. The AIM2 inflammasome, short for 'absent-in-melanoma 2,' is essential in the recognition and defence against self and pathogen associated double-stranded DNA (dsDNA) (Burckstummer et al., 2009; Fernandes-Alnemri et al., 2009). Therefore, this defence mechanism can be simply referred to as a cytosolic dsDNA sensor. DNA sensing by AIM2 is initiated through the recognition of DNA and results in the secretion of mature interleukin (IL)-1β and IL-18, followed by pyroptosis (Gray et al., 2016; Strittmatter et al., 2016). Current research suggests AIM2 participates in the inflammasome by direct binding of pathogen-derived DNA (Strittmatter et al., 2016). After DNA binding, AIM2 forms multimers, allowing it to recruit the adaptor protein ASC and subsequently ASC recruits caspase-1 into the inflammasome complex (**Figure 1**). AIM2 appears to lack sequence specificity due to the fact that it interacts with the sugar-phosphate backbone and does not interact with bases in the major or minor groves (Jin et al., 2012). Thus, AIM2 has the ability to detect any and all DNA in the cytoplasm that is at least 80bp long and is "naked" or not masked by the pathogen in some way (i.e. DNA binding proteins or capsids/membranes) (Jin et al., 2012). As a result, AIM2 is able to respond to pathogen DNA from viruses, bacteria, fungi and parasites if such DNA is naked in the cytoplasm. AIM2 is also capable of responding to transfected human DNA and may play a role in some forms of autoimmunity such as systemic lupus erythematosus (SLE) (Zhang et al., 2013). However, we will only discuss the role for AIM2 in pathogen

detection in this chapter. In addition, we will discuss the host and pathogen encoded proteins involved in activating or supressing AIM2 inflammasome activation.

AIM2 during viral infection

To combat viral pathogens, the innate immune system must communicate the sensing of nucleic acids within the cytoplasm to effectively induce antiviral immune responses. The sensing of nucleic acids during viral infection is particularly important as viruses are diverse in capsid and other protein structures such that they are poor PAMPs. Instead, viruses have conserved nucleic acid structures that serve as PAMPs, either due to unique features of their nucleic acids or due to the unique location of these nucleic acids. For example, healthy cells should not have DNA in the cytoplasm. Thus, any pathogen DNA in the cytoplasm indicates a disease state. AIM2 was initially reported to activate the inflammasome in response to cytoplasmic DNA from Vaccinia virus (VV) or Murine cytomegalovirus (MCMV) (Fernandes-Alnemri et al., 2010; Hornung et al., 2009; Rathinam et al., 2010). More recently, the secondary messenger, 2′3′-cGAMP, which is typically associated with type-I interferon induction, was also showed to play a role in the activation of the AIM2 and NLRP3 inflammasomes during MCMV infection (Swanson et al., 2017). Alternatively, pox viruses possess pyrin only proteins with the ability to inhibit inflammasome activation (Dorfleutner et al., 2007). Recently, human encoded pyrin only protein 3 (POP3) was reported to inhibit the AIM2 inflammasome during VV or MCMV infection and expression of human POP3 in mice, which naturally lack POP3, impaired the immune response to MCMV (Khare et al., 2014). Thus, human cells can regulate the activation of the AIM2 inflammasome via induced expression of POP3, but the importance of this in humans remains to be seen.

Human cytomegalovirus (HCMV) is an enveloped linear double-stranded DNA virus, and member of the *Herpesviridae* family, *Betaherpesvirinae* subfamily. The AIM2 inflammasome is important for control of virus replication in human THP1 monocytes (Huang et al., 2017a). Furthermore, HCMV encoded pUL83 protein inhibits the AIM2 inflammasome, presumably to facilitate virus infection (Huang et al., 2017b). This appeared to be the result of pUL83 interaction with AIM2 that resulted in decreased protein levels of AIM2, pro-caspase-1, and pro-IL1β (Huang et al., 2017b).

Herpes simplex virus 1 (HSV-1) is an enveloped linear double-stranded DNA virus and member of the *Alphaherpesvirinae* subfamily. Mucocutaneous portions of the skin, mainly keratinocytes, are the target of HSV-1 infections, whereas HSV1 becomes latent in nerves of the trigeminal ganglion (Theil et al., 2003). Strittmatter et al. investigated the role of (HSV-1) within keratinocytes (Strittmatter et al., 2016). Here, they found that AIM2 played the key role in the assembly of the inflammasome response (Strittmatter et al., 2016). Their results indicate that IL-1β and IL-18 originating from keratinocytes form a feedback loop that is important for the control of HSV-1 skin infections. However, the epithelial cells must be primed with interferon-γ in order to secrete active forms of pro-inflammatory cytokines during HSV-1 infections (Strittmatter et al., 2016). Their research also suggests that keratinocytes use AIM2 as their sensor, but other cell types, such as primary human monocytes (PHM) or THP1 cells, use NLRP3 as the key sensor and that IFI16 is the key sensor in fibroblasts. Therefore, the PRR responsible for inflammasome activation depends on which cellular model is used in HSV-1 infection (Strittmatter et al., 2016). There are reports of keratinocytes lacking the ability to secrete IL-1β upon HSV-1 infection or that the levels are too low to detect (Milora et al., 2014). This could indicate that HSV-

1 is a weak inflammasome activator or suppresses IL-1β as *Herpesviridae* family members possess many inhibitors of the immune response, suppression of IL-1β production seems likely (Paludan et al., 2011). Strittmatter et al. confirmed that HSV-1 supresses IL-1β production specifically at the posttranscriptional level. Furthermore, HSV-1 inhibits inflammasome activation. However, priming keratinocytes with IFN-γ stimulated caspase-1 activation and secretion of IL-1β and IL-18 (Strittmatter et al., 2016). Finally, HSV-1 VP22 protein was recently reported to inhibit AIM2 inflammasome assembly (Maruzuru et al., 2018). In addition to HSV-1 inhibition of the AIM2 inflammasome, the cellular protein TRIM11 also reduces AIM2 inflammasome activation via autophagic degradation of AIM2 during HSV-1 infection (Liu et al., 2016b). Therefore, host and virus mediated inhibition of AIM2 are involved in regulating inflammasome activation and treatment with IFN-γ to enhance priming of the AIM2 inflammasome may have therapeutic benefit.

Epstein-Barr virus (EBV), also known as Human Herpesvirus 4 (HHV-4) or mononucleosis, is also an enveloped double-stranded DNA virus of the *gammaherpesvirinae* subfamily. Little is known about the activation of the inflammasome in response to primary EBV infections. Epithelial and B cells are the two common cell types infected by EBV. Recently, Torii et al. discovered that EBV infects human monocytes, specifically THP-1 cells and PHM and an inflammasome response develops. Specifically, caspase-dependent IL-1β production occurs within these cells but not within B or T-cell lines (Torii et al., 2017). However, Ansari et al. provides evidence that inflammasome activation does occur in B lymphocytes and epithelial cells, but only during latency (Ansari et al., 2013). To identify the sensor molecule responsible for EBV mediated inflammasome activation in monocytes,

mRNA and protein levels of AIM2 and NLRP3 were examined. Increased levels of AIM2 were observed in EBV-infected THP-1 cells and PHM, whereas levels of NLRP3 did not show remarkable change. Knockdown of AIM2 through small interfering RNA (siRNA) also attenuated caspase-1 activation (Torii et al., 2017). Therefore, the AIM2 inflammasome was involved in the inflammatory response of monocytes to EBV. Further data by Torii et al. showed that serum IL-18 levels were high in patients with acute EBV, suggesting that monocytes and macrophages may play a role in the primary infection or reactivation of EBV, or that some other cell type responds to EBV *in vivo* (Torii et al., 2017). One question that remains is how EBV infects monocytes. Monocytes lack CD21, the primary receptor for EBV, but infection could occur through CD35 and HLA-DR in CD21-decificent cells (Ogembo et al., 2013). Interestingly, it appears that infection of monocytes with EBV is not productive, as EBV BZLF1 gene expression was elevated in EBV-infected THP-1 cells, but EBV DNA replication was not observed (Torii et al., 2017). One possible explanation is that AIM2 inflammasome activation in monocytes results in pyroptosis from successful caspase-1 activation (Torii et al., 2017).

Hepatitis B virus (HBV) is a member of the *Hepadnavirdae* family and is another enveloped, double-stranded DNA virus. Worldwide, HBV poses an imperative health risk. An associated issue to contracting the disease is kidney inflammation, namely glomerulonephritis. This specific condition, known as HBV-GN, is associated with increased expression of AIM2, caspase-1 and IL-1β (Zhen et al., 2014). Through an *in vitro* model with primary human glomerular mesangial (HGM) cells transfected with HBV DNA, enhanced levels of caspase-1, IL-1β and IL-18 were observed. Utilization of siRNA knockdown of AIM2 decreased inflammasome activation (Zhen et al., 2014).

However, it remains to be seen if natural infection also results in AIM2 inflammasome activation, instead of transfection of viral DNA. Immunohistochemistry analysis of human clinical samples provides further evidence to the positive correlation of AIM2, caspase-1, IL-1β, and lL-18, as these were all present in tissue affected by HBV-GN (Zhen et al., 2014). Another study showed through immunohistochemistry of liver biopsies that caspase-1, IL-1β and lL-18 were all elevated in HBV infected patients (Han et al., 2015). Thus, a clear association between the AIM2 inflammasome and HBV infection exists, but further research is needed to decipher the details.

Finally, adenoviruses (AdVs) are non-enveloped linear double-stranded DNA viruses often used for viral transduction or for pseudotyping as vaccines. Interestingly, AdVs do not activate the AIM2 inflammasome unless the cells are first primed through the type I IFN pathway (Liu et al., 2017). Instead, AdVs activate the NLRP3 inflammasome (Barlan et al., 2011a; Barlan et al., 2011b; Muruve et al., 2008). Alternatively, the adenovirus based canarypox virus vector was able to activate the AIM2 inflammasome due to simultaneous type I IFN pathway activation (Liu et al., 2017). Additionally, antibody binding to AdVs following repeated AdV exposure facilitates virus uptake by dendritic cells and subsequent AIM2 inflammasome activation (Eichholz et al., 2016). Thus, AIM2 inflammasome activation to some viruses requires further priming or cytosolic delivery mechanisms.

RNA-based viruses, such as influenza A virus (IAV), are major public health risks around the world. IAV is a member of the *Orthomyxoviridae* family and has an 8 RNA segmented genome. Although an RNA virus, two groups have reported a role for the AIM2 inflammasome during IAV infection in mice. As IAV has a negative-sense single-

stranded RNA genome, the only plausible source of DNA to activate AIM2 is host-derived. Specifically, host DNA released into the lung is recognized by alveolar macrophages, resulting in AIM2 inflammasome activation (Schattgen et al., 2016; Zhang et al., 2017). These two reports have aided our understanding of inflammation by demonstrating a cross-talk between PAMPs and DAMPs through analysis of the release of host DNA into the lung microenvironments from dead cells in response to IAV infection. The endogenous DNA is likely derived from neutrophil extracellular traps (NETs) or necrotic bronchiolar epithelial cells. Levels of dsDNA in mice infected with IAV were increased by bronchiolar lavage (BAL) analysis (Schattgen et al., 2016). Treatment of IAV infected mice with recombinant adeno-associated virus (AAV) that expressed DNase-I reduced the amount of DNA in the BAL (Schattgen et al., 2016). FACS analysis of leukocyte populations within the lungs of AAV-DNase-I treated mice, or *Aim2*$^{-/-}$ mice revealed a significant increase in the number of $CD4^+$ and $CD8^+$ T cells compared to control mice at 5 days-post-infection. In these experiments, host DNA present in the lung microenvironment due to IAV infection activated AIM2, which protected mice from overt inflammation (Schattgen et al., 2016). However, Zhang et al. also reported that AIM2 can respond to host derived DNA in the lungs of IAV infected mice, but reported that *Aim2*$^{-/-}$ mice survive IAV infection better than WT mice (Zhang et al., 2017). Improved survival in *Aim2*$^{-/-}$ mice was associated with increased monocytes and decreased neutrophils in the lungs (Zhang et al., 2017). In both reports, viral load within the lungs of WT and *Aim2*$^{-/-}$ mice were not different (Schattgen et al., 2016; Zhang et al., 2017). This suggests that AIM2 does not directly impact IAV replication. However, as each group reported different findings with regard to survival, more research is needed to understand the role for AIM2 during IAV and other RNA virus infections. As DNA in this setting is host derived, it would be a DAMP instead of

a PAMPs, and future research into host DAMPs and their role during infection is needed.

Another RNA virus, enterovirus A71 (EV-A71), which is a positive-sense single-stranded RNA virus, may also require AIM2 for induction of neuronal pathology. Yogarajah et al. observed that AIM2, caspase-1, and IL-1β expression were all elevated in EV-A71 infected neurons and caspase-1 activation occurred (Yogarajah et al., 2017, 2018). Furthermore, siRNA mediated knockdown of AIM2 impaired cell death and resulted in increased virus replication (Yogarajah et al., 2017). However, it remains to be seen how AIM2 is activated during EV-A71 infection, as this is an RNA virus.

AIM2 and bacteria

Shifting to bacterial infections, the AIM2 inflammasome mediates recognition of many cytosolic bacteria. AIM2 activation by *Francisella tularensis* subspecies *novicida* (*F. novicida)* and *Listeria monocytogenes* (*L. monocytogenes*) was reported nearly a decade ago (Fernandes-Alnemri et al., 2010; Jones et al., 2010; Rathinam et al., 2010). Importantly, *F. novicida* is only sensed by the AIM2 inflammasome, whereas *L. monocytogenes* is also sensed by the NLRP3 and NLRC4 inflammasomes (Kim et al., 2010; Rathinam et al., 2010; Tsuchiya et al., 2010; Warren et al., 2010; Wu et al., 2010). The obvious connection is that both bacteria enter the cytoplasm and DNA is sensed by AIM2 to induce inflammasome activation, and that *F. novicida* lacks a flagellum to activate NLRC4. However, it is not clear how *F. novicida* avoids NLRP3 activation. One possible explanation comes from another bacterium which also activates AIM2 and NLRP3, namely *Legionella pneumophila.* During *Legionella*

pneumophila infection, inflammasome activation is sequential, where AIM2 initially forms inflammasomes in response to bacterial DNA in the cytoplasm and activates caspase-11 resulting in cell membrane damage. This then allows for NLRP3 inflammasome activation (Cunha et al., 2017). Finally, in the absence of caspase-1, AIM2 and ASC activate caspase-8 mediated apoptosis to inhibit bacterial replication (Pierini et al., 2012). These findings demonstrate that differential responses of the immune system to specific pathogens can be mediated by inflammasome activation of different caspases and further research in this area is needed to better understand these mechanisms.

In order for AIM2 to detect *F. novicida*, *L. monocytogenes*, and presumably other bacteria in the cytoplasm, bacterial lysis is required. Some genetically modified strains of *F. novicida* and *L. monocytogenes* can lyse more easily, resulting in improved AIM2 activation (Peng et al., 2011; Sauer et al., 2010). Even WT bacteria lyse periodically, resulting in low levels of AIM2 inflammasome activation (Sauer et al., 2010). This begs the question of whether the AIM2 response is a passive or active, host-driven, immune mechanism. Numerous reports indicate that type-I interferon signalling is required for robust AIM2 inflammasome activation during *F. novicida* (Belhocine and Monack, 2012; Fernandes-Alnemri et al., 2010; Jones et al., 2010) and even *Streptococcus pneumoniae* infection (Fang et al., 2014). This would suggest some priming or cell activation event is required for complete AIM2 inflammasome activation. Several studies conclusively demonstrate that type-I interferon signalling upregulates guanylate-binding proteins (GBPs). Specifically, GBP2 and GBP5 are key AIM2 activators during *F.* novicida infection (Man et al., 2015; Meunier et al., 2015; Wallet et al., 2017). When *F. novicida* becomes cytosolic, promotion of bacteriolysis occurs.

This is achieved through GBP mediated localization of another protein, IRGB10, to the bacterial membrane (Man et al., 2015; Meunier et al., 2015). There, IRGB10 inserts into bacterial membranes and destabilizes them, releasing bacterial DNA and other ligands into the cytoplasm (Man et al., 2016). A similar requirement for GBPs for AIM2 inflammasome activation during infection with *Brucella abortus* and *Legionella pneumophila* was also reported (Costa Franco et al., 2018; Liu et al., 2018). However, endogenous levels of GBPs were sufficient for bacterial ligand release in *Legionella pneumophila* infected cells (Liu et al., 2018). GBPs are also important for early AIM2 activation during infection with *Chlamydia muridarum* and *Chlamydia trachomatis*, but decrease in importance over time, suggesting other mechanisms also play a role in AIM2 activation with *Chlamydia* (Finethy et al., 2015). Finally, it must be noted that AIM2 activation via transfection of synthetic DNA or during DNA viral infections is independent of this GBP pathway (Man et al., 2015) and that GBPs possess other, inflammasome independent, mechanisms for inhibiting bacterial growth (Wallet et al., 2017).

Several *Mycobacterium* species also activate the AIM2 inflammasome, including *Mycobacterium tuberculosis, M. smegmatis* and *M. bovis* (Saiga et al., 2012; Shah et al., 2013; Yang et al., 2013b). Interestingly, virulent strains of *M. tuberculosis* failed to activate the AIM2 inflammasome despite the presence of bacterial DNA in the cytoplasm (Shah et al., 2013). Accordingly, the ESX-1 protein of virulent *M. tuberculosis* inhibited type-I interferon mediated induction of AIM2 and potentially directly inhibits AIM2 inflammasome activation as well (Shah et al., 2013). On the other hand, AIM2 may competitively inhibit type-I interferon and autophagy responses

during infection with *M. bovis* by binding to bacterial DNA and preventing detection of bacterial DNA by other cytoplasmic DNA sensors like STING (Liu et al., 2016a).

In conclusion, much research has been done to understand the role for the AIM2 inflammasome during bacterial infection and a myriad of other bacterial species are able to activate the AIM2 inflammasome. These include *Porphyromonas gingivalis* (Park et al., 2014), *Staphylococcus aureus* infection of the central nervous system (Hanamsagar et al., 2014), and *Brucella abortus* (Gomes et al., 2013; Miraglia et al., 2016). However, as the recent discovery of GBPs indicates, there is still much we do not understand about this inflammasome.

AIM2 and fungi

Fungal pathogens are extremely interesting in their virulence patterns. Most fungal pathogens do not release their genetic material within the cytosol during infection (Niemiec et al., 2017). Therefore, the number of fungal pathogens activating the AIM2 inflammasome is scarce to our knowledge. However, *Aspergillus fumigatus* is gaining attention in recent years due to its involvement in infecting as an opportunistic pathogen. This fungus is saprophytic and ubiquitous, making contact with immunocompromised individuals inevitable. There is limited research pertaining to the involvement of *A. fumigatus* and its affects with the cytosolic dsDNA sensor, AIM2. However, AIM2 and NLRP3 activation form a dual cytoplasmic surveillance system that orchestrates responses against *A. fumigatus* infection (Karki et al., 2015). Both AIM2 and NLRP3 engage the inflammasome to trigger innate immune responses against *A. fumigatus* infections. Mice lacking AIM2 and NLRP3 are observed to be hyper susceptible to invasive aspergillosis (Karki et al., 2015). This is due to a failure

to confine *Aspergillus* hyphae in the lung and leads to widespread hyphal dissemination to lung blood vessels. Both of these inflammasome receptors activate caspase-1 and induce processing of IL-1β and IL-18 by recruiting caspase-8 to the same inflammasome complex. From there, IL-1β, IL-18, caspase-1, caspase-8, and FADD are crucial for preventing *Aspergillus*-induced mortality (Karki et al., 2015). Together, these results unveiled a dual cytoplasmic sensing mechanism governed by AIM2 and NLRP3 to orchestrate robust host responses to *A. fumigatus*. AIM2 and NLRP3 play redundant roles in driving robust inflammasome activation to mediate the secretion of IL-1β and IL-18 in response to *Aspergillus* infection (Karki et al., 2015). Interestingly, *Candida albicans* activates NLRP3 exclusively (Wellington et al., 2014). This is possibly due to the failure of this microorganism to release its DNA into the host cytosol to activate AIM2, or this specific fungus contains a virulence factor that mediates its evasion from this surveillance mechanism. Overall, the extracellular release of *A. fumigatus* DNA due to germination of this fungus may activate the AIM2 inflammasome (Karki et al., 2015).

AIM2 and parasites

Similar to AIM2 and fungi, there exists limited data and knowledge about the correspondence of parasites and the AIM2 inflammasome. *Plasmodium falciparum* is a causative agent of malaria. As malaria continues to be one of the most devastating global health concerns in human history, understanding the immune response to this infection is critical for improving treatment options. Kalantari et al. describes the possible role for AIM2 during *Plasmodium falciparum* infection (Kalantari et al., 2014). They discovered that the haemoglobin by-product hemozoin can carry parasite DNA into the cytoplasm and induce both NLRP3 and AIM2 inflammasome activation

(Kalantari et al., 2014). In general, a pro-inflammatory cytokine storm results when parasitized red blood cell rupture, and IL-1β has been linked to this condition. Because of its highly synergistic nature, IL-1β can modify the expression and actions of other modulators of the immune system (Kalantari et al., 2014).

Overall, AIM2 inflammasome activation is essential for host defence and clearance of intracellular associated pathogens including bacteria, viruses, fungi and parasites. Furthermore, a variety of pathogen and host encoded modulators or AIM2 activation can affect pathogen growth and/or inflammation and tissue damage. Therefore, the importance of AIM2 inflammasome activation deserves to be investigated further.

NLRP3: the omniscient inflammasome

To say that NLRP3 responds to 'numerous' stimuli would be under-statement. The NLRP3 inflammasome reacts to 'stuff', varying from reactive oxygen species, potassium efflux, bacterial pore-forming toxins and non-infectious crystals (Place and Kanneganti, 2018). These stimuli are the usual basis for the activation of the NLRP3 inflammasome pathway. However, as NLRP3 has a reported role in virtually every infectious or non-infectious disease, we must limit our discussion here to a select number of publications that indicate NLRP3 may possess the ability to directly, or through adaptor proteins, indirectly detect microbial or self-nucleic acids. We will not discuss the myriad of papers involving nucleic acid mediated priming of the NLRP3 inflammasome.

NLRP3 detection of pathogen nucleic acids

The idea that the NLRP3 inflammasome could respond to pathogen nucleic acids was first reported by Kanneganti and colleagues (Kanneganti et al., 2006a; Kanneganti et al., 2006b) (**Figure 2**). Infection of macrophages with enterohemorrhagic *Escherichia coli* by another group also activated the NLRP3 inflammasome through the presence of RNA:DNA hybrids in the cytoplasm of infected cells that localized to NLRP3 inflammasome (Kailasan Vanaja et al., 2014). Furthermore, various RNA species (mRNA, tRNA, rRNA) were found to activate the NLRP3 inflammasome (Sha et al., 2014). However, no direct mechanism for NLRP3 mediated nucleic acid sensing was evident in any of these reports. Another report by Gupta et al. showed that RNA from Group B Streptococcus (GBS) could activate NLRP3, and bacterial RNA could be immunoprecipitated with NLRP3 (Gupta et al., 2014). However, this does not rule out the potential for an intermediate RNA binding protein associated with NLRP3. The missing pieces to this puzzle may have been discovered with DHX33 and DDX19A, both members of the DExD/H-box helicase family. Both proteins can form a complex with NLRP3 in human or porcine monocyte or macrophages, respectively, in response to cytoplasmic viral and bacterial RNA (**Figure 2**). These interactions result in inflammasome activation (Li et al., 2015; Mitoma et al., 2013). Specificity of DHX33 for pathogen RNA was based on the presence of double stranded RNA in the cytoplasm (Mitoma et al., 2013). Alternatively DDX19A recognized the 5’ and 3’ untranslated regions (UTRs) of porcine reproductive and respiration syndrome virus (PRRSV) (Li et al., 2015). Furthermore, DHX33 mediated activation of NLRP3 is regulated by TRIM33 through ubiquitination of DHX33 (Weng et al., 2014), thus allowing control of this inflammatory pathway. Lastly, the 2',5'-oligoadenylate (2-5A) synthetase (OAS)/RNase L pathway can process viral RNA and generate ligands for DHX33 to facilitate NLRP3 inflammasome activation (Chakrabarti et al., 2015).

NLRP3 detection of self-nucleic acids during infection

Detection of self-DNA by AIM2 during pathogenic infection was previously discussed for infection with IAV above. However, mitochondrial DNA (mtDNA) is another potential source of host derived nucleic acid with the ability to activate the inflammasome (**Figure 2**). Intriguingly, mtDNA does not appear to activate the AIM2 inflammasome, unless it is transfected into cells (Shimada et al., 2012). Instead, cellular damage during infection with *Chlamydia pneumoniae* or treatment with LPS+ATP resulted in the release of mtDNA into the cytoplasm where it could be immunoprecipitated with NLRP3 and lead to inflammasome activation (Shimada et al., 2012). Accumulation of damaged mitochondria due to impaired autophagy further allows for mtDNA mediated NLRP3 inflammasome activation (Nakahira et al., 2011). More recently, new mtDNA synthesis mediated by CMPK2 was shown to be required for NLRP3 activation (Zhong et al., 2018). However, it remains to be determined if this is a direct NLRP3 interaction, or if some intermediate protein facilitates this interaction. Overall, the studies discussed here suggest that self mtDNA deserves further examination as a DAMP induced by pathogen invasion with the potential for NLRP3 inflammasome activation.

IFI16 assembles an inflammasome in the nucleus

Inflammasomes are mainly assembled by members of the NLR family, which upon activation by apt stimuli result in cytoplasmic organization of the supramolecular complex with critical roles in protection against cytosolic pathogens. Whether inflammasomes can assemble in the nucleus and impart protection against nuclear

replicating pathogens wasn't known until the role of the DNA sensor interferon gamma-inducible protein 16 (IFI16) was elucidated (Kerur et al., 2011).

IFI16 is predominantly a nuclear protein and belongs to the interferon (IFN)-inducible p200 (also known as PYHIN) family. In mice, the p200 gene family includes p202a, p202b, p203, p204, MNDAL, and AIM2, while in humans, the gene family is represented by IFI16, MNDA, AIM2, and IFIX (Zhao et al., 2015). The p200 family members consist of at least one partially conserved repeat of 200-amino acid residues (the HIN-200 domain) towards their C-terminus and all of them (except p202a and p202b in mice) have a PYRIN domain (PYD) at their N-terminus allowing homotypic interactions with other PYD-containing proteins (Ludlow et al., 2005). On the other hand, the HIN-200 domain binds DNA and the IFI16 protein in particular contains two repeats of this domain separated by a spacer rich in serine-threonine-proline. Human IFI16 is regarded as a murine p204 homolog based on similar functions and closely related domain structure, however, further functional evidence supporting this is required (Cridland et al., 2012). The role and mechanistic details of IFI16 in type I interferon production has been established recently. In response to viral genomes or transfected DNA, IFI16 directly associates with DNA motifs through the HIN-200 domains leading to the oligomerization of IFI16 and activation of downstream STING, causing a conformational change in the STING dimer. STING subsequently associates with TANK binding kinase 1 (TBK1) leading to phosphorylation of STING and recruitment and phosphorylation of interferon regulatory factor 3 (IRF3) (Dobbs et al., 2015; Liu et al., 2015). Phosphorylated IRF3 then enters the nucleus triggering the type I interferon gene program **(Figure 3)**. Previous studies have also implicated IFI16 in cell cycle regulation, differentiation and apoptosis and these are discussed in detail

elsewhere (Aglipay et al., 2003; Brunette et al., 2012; Luan et al., 2008; Xin et al., 2003; Zhang et al., 2008; Zhao et al., 2015).

The potential clue to investigate the role of IFI16 in inflammasome formation came from the presence of the PYD and HIN-200 domains in IFI16 (Xiao, 2015). Similar to IFI16, the DNA sensor AIM2 also contains both PYD and HIN-200 domains and assembles an inflammasome in response to dsDNA. The structural and functional similarities between AIM2, IFI16 and other DNA receptors prompted conception of a new family of innate DNA sensors called AIM2-like receptors (ALRs). Consistent with its localization, IFI16 was observed to trigger inflammasome assembly in response to nuclear replicating Kaposi sarcoma-associated herpesvirus (KSHV) infection of primary HMVEC-d endothelial cells (Kerur et al., 2011) **(Figure 3)**. The involvement of other DNA receptors was ruled out, either because they are not expressed in HMVEC-d cells (e.g. MNDA), or because they are predominantly localised in the cytosol (e.g. AIM2) and are thus unable to respond to KSHV infection. Contrary to monocytes and macrophages where caspase-1 is predominantly cytosolic, HMVEC-d cells expressed ASC and pro-caspase-1 predominantly in the nuclei of uninfected cells, which upon KSHV infection assembled the nuclear IFI16 inflammasome (Kerur et al., 2011). Of note, the complex is also observed in the cytosol. However, there is evidence that the IFI16 inflammasome first assembles in the nucleus and subsequently redistributes to the cytosol. In agreement, the active p20 band of caspase-1 was observed in nuclear fractions of infected cells as early as 2h p.i., although it was also detectable in the cytoplasm at the earliest time examined (Kerur et al., 2011). Moreover, IFI16 directly colocalized with KSHV genomes in the nuclei of infected endothelial cells. Similarly, immunofluorescent studies demonstrated ASC and caspase-1 aggregates in the

perinuclear area at later times p.i. revealing the dynamic nature of the IFI16 assembled inflammasome between subcellular compartments (Kerur et al., 2011). As IFI16 is also observed in the cytoplasm (Unterholzner et al., 2010), it is unclear what precludes inflammasome formation in the cytoplasm, but this may be dependent on the spatio-temporal expression and localization of IFI16 in the cell type involved, proximity to viral DNA, and/or any necessary IFI16 post-translational modifications. Nonetheless, the relocation of IFI16 to the cytoplasm provides a fail-safe mechanism to avert overt caspase activity in the nucleus. Moreover, these findings suggest that IFI16 complements other plasma membrane and cytosolic pathogen sensors in the nucleus signifying how the host cells have reinforced each cellular compartment to evade pathogen invasion.

The physiological significance of the IFI16 inflammasome in humans demands further investigation. In a clinical study conducted with mucosal biopsies obtained from two different cohorts of patients with inflammatory bowel disease (IBD), the mRNA expression of both AIM2 and IFI16 was found significantly upregulated in the inflamed colon of active patients as compared to noninflamed colon of controls and inactive patients with IBD (Vanhove et al., 2015). The transcriptional data correlated well with immunoblotting studies exhibiting increased protein expression of AIM2 and IFI16 in the colonic mucosa of active patients (Vanhove et al., 2015). These findings were independently verified by a study which confirmed elevated expression by immunohistochemistry of IFI16 in intestinal samples from both ulcerative colitis and Crohn's disease patients (Caneparo et al., 2016). Moreover, the latter study revealed elevated IFI16 expression limited to the intestinal epithelial cells of IBD patients. Although data was not included, IFI16 staining was conspicuously absent in intestinal

epithelial cells from other non-IBD inflammatory conditions (Caneparo et al., 2016). Increased caspase-1 and the inflammasome downstream effector HMGB1 has also been observed in the epithelial compartment of the colonic mucosa from patients with active disease (Vanhove et al., 2015). Additional studies in genetic models of IFI16 are required to directly implicate the role of IFI16 in disease pathogenesis. As inflammasomes are critically involved in the pathogenesis of IBD, improved understanding in this area could have important implications in developing more personalized therapies for IBD patients.

IFI16-mediated pyroptosis depletes CD4$^+$ T cells during HIV infection

Activation of the inflammasome is almost always followed by an inflammatory form of cell death, pyroptosis. Pyroptosis of the infected cells results in destruction of the pathogen niche; however, this is also accompanied by tissue damage because of the nature of this form of cell death (Kayagaki et al., 2015; Lupfer and Anand, 2016). Depletion of quiescent 'bystander' CD4$^+$ T cells is the principal driver of AIDS during HIV infection. The bystander CD4$^+$ T cells are non-permissive to HIV infection and undergo abortive infection characterized by cytosolic accumulation of incomplete HIV reverse transcripts (Doitsh et al., 2010). IFI16 recognises these reverse transcripts leading to caspase-1 activation and pyroptosis followed by LDH secretion (Monroe et al., 2014). In agreement, shRNA-based knockdown of IFI16 rescued CD4$^+$ T cells cocultured with T cells infected with a HIV-GFP reporter virus from cell death. By contrast, knock down of AIM2 and STING did not have any appreciable effect on LDH release and thus pyroptosis. Of note, cell death could be rescued in control cells upon addition of a non-nucleoside reverse transcriptase inhibitor, efavirenz, thereby supporting the notion that the CD4$^+$ T cell depletion results from abortive infection and

not viral integration (Doitsh et al., 2010; Monroe et al., 2014). Moreover, IFI16 knock-down not only decreased active caspase-1 levels (determined by FLICA), but also IFN-β induction, suggesting an upstream role of IFI16 in triggering both these pathways in $CD4^+$ T cells. Altogether, these findings suggest that IFI16 induces caspase-1 mediated pyroptosis in abortively infected $CD4^+$ T cells thereby highlighting the detrimental consequences of inflammasome activity during AIDS progression. The precise localization of IFI16 and whether the assembled inflammasome redistributes between subcellular compartments during HIV infection is not known.

Acetylation of IFI16 regulates cytoplasmic and nuclear shuttling

Despite being predominantly nuclear at steady-state levels, IFI16 can unexpectedly sense pathogenic DNA in both the nucleus and the cytoplasm suggesting either that a fraction of IFI16 is always cytosolic or that IFI16 is distributed from the nucleus to the cytosol by a post-translational modification. The latter scenario is supported by two independent studies proposing that the acetylation of IFI16 is critical for nuclear and cytoplasmic shuttling (Ansari et al., 2015; Li et al., 2012). However, precisely where IFI16 is acetylated is less clear. Li et. al. revealed the presence of an evolutionarily conserved multipartite nuclear localization signal (NLS) in IFI16 and proposed that the acetylation of IFI16 NLS in macrophages restricts the protein to the cytoplasm by inhibiting nuclear import (Li et al., 2012). Thus, treatment with the broad-spectrum histone deacetylase (HDAC) inhibitor trichostatin A triggered accumulation of cytoplasmic IFI16. Subsequent experiments suggested the involvement of acetyltransferase p300 in regulating IFI16 localization. In agreement, overexpressing p300 displayed a striking cytoplasmic accumulation of IFI16-GFP. A study by Ansari and colleagues confirmed the above findings but revealed that IFI16 modification by

acetyltransferase p300 occurred in the nucleus immediately following the recognition of herpesvirus genomes (Ansari et al., 2015). Acetylation of IFI16 preceded IFI16-ASC interaction in the nucleus before the assembled IFI16 inflammasome translocated to the cytoplasm resulting in caspase-1 activation and IFN-β production. In agreement, IFI16 acetylation remained unaffected by either ASC or STING knockdown. Furthermore, IFI16 remained unmodified and contained within nucleus upon cytoplasm restricted vaccinia virus infection, highlighting both that acetylation occurred in the nucleus and that the modification is necessary for IFI16 translocation to the cytoplasm. However, acetylation was not found to be required for DNA sensing and IFI16 physically associated with KSHV and HSV-1 genomes regardless of the presence of acetylation inhibitor (Ansari et al., 2015). Further studies should address whether IFI16 acetylation is common to other model systems and to decipher the precise compartment where acetylation occurs. Regardless, these findings establish acetylation as a molecular toggle regulating IFI16 distribution.

Viral evasion of the IFI16 inflammasome

Activation of the innate immune responses including inflammasomes is essential to protect against pathogens. In turn, viruses have co-opted several mechanisms to establish productive infection in their host cells. HSV-1 establishes life-long latency in infected cells. Similar to KSHV infection, HSV-1 infection of human skin fibroblasts activates the IFI16 inflammasome followed by relocalization of the receptor complex to the cytoplasm (Johnson et al., 2013). Additionally, in contrast to KSHV, HSV-1 infection also activates the NLRP3 inflammasome. However, IFI16 is distinctly targeted for degradation at later times post infection in a manner dependent on ICP0, an immediate early protein of HSV-1. While the expression of NLRP3 and ASC remained unaltered, caspase-1 was also found ‘trapped’ in actin clusters thereby

blocking the overall activity of both the NLRP3 and IFI16 inflammasomes (Johnson et al., 2013). The above findings are independently confirmed by another study which demonstrated that expression of viral ICP0 protein in the nucleus causes nuclear relocalization and degradation of IFI16 thereby inhibiting the STING-IRF3 signaling pathway (Orzalli et al., 2012). However, the latter study did not observe any detectable change in IFI16 localization and the receptor remained nuclear restricted in control and HSV-1 infected human skin fibroblasts (Orzalli et al., 2012). These studies established that HSV-1 ICP0 protein targets IFI16 and caspase-1 to inhibit the inflammasome pathway. IFI16 also acts as a nuclear DNA sensor for human cytomegalovirus (HCMV) in human foreskin fibroblasts (HFFs) triggering the expression of antiviral cytokines via the STING-TBK1-IRF3 signaling pathway. However, the HCMV tegument protein pUL83 (also known as phosphoprotein pp65) interacts with the pyrin domain of IFI16 thereby blocking the oligomerization and induction of antiviral responses (Li et al., 2013). Accordingly, infection with a HCMV mutant strain unable to make pUL83-encoded pp65 enhanced IFN-β production as compared to HFFs infected with the wild-type strain (Biolatti et al., 2018). Of interest, in THP-1 derived macrophages, interaction between recombinant pUL83 and AIM2 has been demonstrated. This has been suggested to reduce the activation of the AIM2 inflammasome upon poly(dA:dT) transfection in a recombinant inflammasome system assembled in HEK293T cells (Huang et al., 2017b). These latter findings need confirmation but would have wider implications if the tegument protein indeed affects inflammasome activation.

IFI16 differentiates self from non-self dsDNA

While the nuclear localization of DNA sensor IFI16 is advantageous in initiating rapid responses to nuclear pathogens, the receptor must also faithfully differentiate between ‘self’ and ‘non-self’ DNA. Crystallographic and binding studies on AIM2 and isolated HIN200 domains of IFI16 suggested that IFI16 assumes an autoinhibited conformation in which the DNA binding HIN200 domain is blocked by the PYD domain which is only displaced upon sensing foreign DNA (Jin et al., 2012). Subsequently, IFI16 one-dimensionally tracks long stretches of exposed foreign dsDNA to assemble into supramolecular signaling platforms (Stratmann et al., 2015). The initiation of IFI16 assembly requires at least 4 molecules and the optimal oligomeric assembly is only reached in the presence of 10 IFI16 molecules with a dsDNA-binding footprint of ~15bp for each IFI16 (Stratmann et al., 2015). The scanning of the exposed DNA is believed to increase the probability of encountering other IFI16 molecules required for IFI16 filament formation (Morrone et al., 2014). At the same time, however, the targeting of ‘self’ DNA is averted because of the presence of host DNA into tightly packed nucleosomes which do not leave long DNA stretches unexposed, a prerequisite for IFI16 DNA tracking and ensuing filament formation (Morrone et al., 2014; Stratmann et al., 2015). Thus, IFI16 molecules cluster into distinct protein filaments only on exposed foreign DNA. Unexpectedly, the HIN200 domain of IFI16 binds DNA with weak affinity without facilitating filament formation. By contrast, the IFI16 PYD domain plays a positive role in DNA binding and drives the filament assembly (Morrone et al., 2014). Furthermore, the surface residues in the PYD domain that enable cooperative DNA binding are conserved suggesting a common strategy likely used by other ALRs (Morrone et al., 2014). These studies thus reveal how IFI16 differentiates friend from foe and highlight the property by which even a single molecule of non-self DNA can be rapidly engaged by IFI16.

NLRP9b: The newest nucleic acid sensing inflammasome

As discussed above, NLRP3 can be activated in response to nucleic acids from pathogens through interaction with several helicase proteins. Similarly, NLRP9b is activated in response to short double stranded RNA fragments (dsRNA) during intestinal infection with rotavirus, but dsRNA does not directly activate NLRP9b. Instead, dsRNA is detected by the helicase DHX9, which subsequently binds to and activates NLRP9b to activate the inflammasome (Zhu et al., 2017). Mice deficient in NLRP9b had higher viral loads, more diarrhoea, and increased destruction of intestinal villi compared to WT controls (Zhu et al., 2017). Interestingly, pyroptosis mediated by NLRP9b inflammasome induced gasdermin D cleavage was responsible for control of rotavirus replication, whereas IL-18 produced by the inflammasome was dispensable (Zhu et al., 2017). Therefore, NLRP9b appears to be a true nucleic acid sensing inflammasome with the help of DHX9, but it remains to be seen how many other pathogens induce NLRP9b, or if it specific only to rotavirus.

Nucleic Acid Sensing Inflammasomes in Autoinflammation and Autoimmunity

Our discussion thus far has focussed on the role for the inflammasomes during infectious disease. However, the ability of some nucleic acid sensing inflammasomes to detect pathogen or mitochondrial DNA in an apparently sequence independent manner poses a risk for unintentionally detecting self-DNA. In the case of NLRP3, there are so many potential activators that determining the importance of mtDNA during *in vivo* disease models is experimentally difficult. Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis are autoinflammatory conditions associated with obesity, glucose insensitivity or other dysregulated metabolic conditions. Two reports now suggest that NLRP3 sensing of mtDNA is involved in these diseases and

is associated with oxidized or increased presence of some fatty acids (Pan et al., 2018; Schuster et al., 2018). In a separate model, mitochondrial damage and release of mtDNA was associated with NLRP3 inflammasome activation during type-1-diabetes (Carlos et al., 2017). Thus, mtDNA mediated activation of NLRP3 can lead to autoinflammatory or autoimmune diseases.

The ability of AIM2 to distinguish friend from foe is based on the location of DNA. In general, host DNA is in the nucleus or packaged in apoptotic bodies and removed by phagocytosis. However, during acute pancreatitis, inflammation results from premature activation of digestive enzymes in the pancreas instead of the intestines. Essentially, the pancreas begins digesting itself resulting in release of nucleic acids (Shah et al., 2009). Deletion of *Aim2* was able to protect mice from tissue damage and inflammation during acute pancreatitis, suggesting that nucleic acids from damaged tissues are somehow internalized into neighbouring cells, or immune cells entering the pancreas, resulting in inflammasome activation (Kang et al., 2016). In another model, *Aim2*$^{-/-}$ mice are protected from the damaging effects of systemic lupus erythematosus (SLE or lupus). During SLE, the immune system responds to nucleic acids or nucleosomes and produces autoantibodies to these entities. Interestingly, *Aim2*$^{-/-}$ mice do not develop the clinical signs of SLE, such as glomerulonephritis, but they still develop autoantibodies (Baum et al., 2015; Jakobs et al., 2015). This indicates that the presence of autoantibodies is not the only issue involved in SLE pathology, and that innate immune signaling and inflammation mediated by nucleic acid sensors such as AIM2 is essential for disease progression (Baum et al., 2015; Jakobs et al., 2015; Zhang et al., 2013). The question here is, how does self-DNA in SLE patients get into the cytoplasm and activate AIM2? One mouse model of SLE is a double knockout for

DNaseII$^{-/-}$ and *Ifnar*$^{-/-}$ (Kawane et al., 2006). DNase II is present in phagosomes where it degrades the DNA from phagocytosed apoptotic bodies or extracellular DNA. In *DNaseII*$^{-/-}$ mice, DNA is not degraded and presumably has the chance to move from the phagosome into the cytoplasm (Monteith et al., 2016). Thus, the ability of AIM2 to distinguish between self and pathogen DNA relies on the ability of the host to keep self-DNA out of the cytoplasm. If DNase II cannot degrade DNA from extracellular sources, then it eventually enters the cytoplasm and activates AIM2. Similarly, the sequestration of mtDNA in the mitochondria limits NLRP3 activation, and only under chronic cellular or metabolic stress does mtDNA escape into the cytoplasm and activate NLRP3.

IFNs are widely recognized to trigger and accelerate the pathogenesis and perpetuation of autoimmune responses. Studies have demonstrated that IFN-inducing IFI16 levels are enhanced in the sera of patients affected by systemic autoimmune diseases including SLE, systemic sclerosis, rheumatoid arthritis, and Sjogren's syndrome (SS) (Alunno et al., 2015; Gugliesi et al., 2013). Particularly, IFI16 has been relatively well characterized in SS where its' levels are elevated and it serves as an autoantigen. Consequently, the levels of both IFI16 and anti-IFI16 antibodies are dominant in the sera of SS patients and are associated with severe outcomes (Alunno et al., 2015). How IFI16 becomes extracellular and adds to the initiation and progression of autoimmune diseases is not fully understood. It has been suggested that IFI16 mislocalization from its' predominantly nuclear site may be involved in initiating secretion to the extracellular milieu (Bawadekar et al., 2015). This may be achieved by tissue injury/apoptosis or inflammasome-induced pyroptosis followed by IFI16 release via exosomes. Increased expression and cytoplasmic mislocalization of IFI16 has been reported in acinar and duct epithelial cells of labial salivary glands of

SS patients (Alunno et al., 2015; Antiochos et al., 2018). In these cells, IFI16 was observed to exist in filamentous structures in the cytoplasm (Antiochos et al., 2018). This filamentous nature of IFI16 required its' association with dsDNA in cultured epithelial cells. Though DNA nucleases could access IFI16-bound dsDNA and degrade it, this did not result in dissociation of IFI16 filaments suggesting the persistence of IFI16 filaments once formed (Antiochos et al., 2018). Moreover, human anti-IFI16-positive SS sera immunoprecipitated filamentous dsDNA-bound IFI16 approximately two- to three-fold more strongly suggesting that autoantibodies preferred epitopes selectively exposed in filamentous IFI16 (Antiochos et al., 2018). These findings outline potential mechanisms and hint that dsDNA-induced oligomerization of IFI16 and subsequent release determines IFI16's status as an antigen in systemic autoimmune diseases.

Conclusions

The immune cells are armed with a limited set of PRRs to respond to diverse PAMPs and DAMPs. Inflammasome forming PPRs have gained tremendous attention in recent years because of the numerous infectious and sterile stimuli they can respond to thereby implicating them in varied diseases. Nucleic acid sensing PRRs including those that assemble inflammasomes are clearly important in maintaining immune homeostasis as they have the obligation to respond to pathogen DNA while differentiating it from hosts' own 'self' DNA. Understanding how these PRRs recognize nucleic acids and maintain specificity is imperative to gain improved realisation for translational purposes.

Mammalian cells display a variety of nucleic acid receptors and three of these (AIM2, NLRP3, and IFI16) are known to establish inflammasomes. While the broad functions of these PRRs are generally known, we still have major gaps in our understanding of what is actually recognized, especially since few structural differences exist between pathogen and self DNA. Clearly, this is not of relevance for NLRP3, as no nucleic acid stimuli appears to bind directly to this receptor. However, some progress has been made in deciphering the principles that AIM2 and IFI16 follow in nucleic acid sensing. Of note, both AIM2 and IFI16 lack sequence specificity. AIM2 recognizes any dsDNA of approximately 80bp in length, so far as it is 'naked' or not masked by the pathogen. In the same way, IFI16 needs long stretches of exposed DNA, which is not tightly packed as in host nucleosomes. While they faithfully respond to their cognate ligands most of the time, any aberrant activity of these PRRs is associated with autoimmune diseases including systemic lupus erythematosus and Sjögren syndrome.

In order to completely appreciate the biology of nucleic acid sensing inflammasome forming PRRs, there is a need to conduct cell type and tissue specific studies in relevant disease models. IFI16, and the assembled inflammasome, shuttles between different compartments; therefore, there is a need to decrypt the precise functions of nucleic acid sensing PRRs in distinct compartments. Such studies should also aid in determining how pathogens may evade inflammasomes. In the evolutionary arms race, pathogens have co-opted advanced mechanisms to counter host strategies. Therefore, it is not unlikely that pathogens recruit histone proteins to their DNA to uniquely evade inflammasome recognition. Furthermore, more animal and clinical studies are needed to unravel the specific mechanisms in a physiological setting to make clinical applications possible. The improved knowledge of the higher order

biochemical and functional details of how inflammasomes sense, oligomerize, and assemble on nucleic acids should help to effectively induce or restore antiviral immunity. Moreover, these insights will likely lead to new treatment avenues for autoimmune diseases. Future studies in this area will help to translate these discoveries into practice.

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Figure Legends

Figure 1: AIM2 inflammasome activation mechanisms

Absent in melanoma 2 (AIM2) is present in an inactive conformation in the cytoplasm of cells. Upon binding to cytoplasmic DNA in a sequence independent manner, AIM2 adopts an open, active conformation that allows for binding to the adaptor protein ASC, which bids to caspase-1. This complete inflammasome allows for activation of caspase-1 and proteolytic processing and activation of the cytokines IL-1β and IL-18. The DNA from some pathogens must be released from inside the pathogen into the cytoplasm. Guanylate binding proteins (GBPs) and IRGB10 are induced by the type I interferon response. GBPs and IRGB10 then bind to pathogens and disrupt the pathogen membranes to facilitate release of pathogen DNA into the cytoplasm where it can activate AIM2.

Figure 2: NLRP3 response to cytoplasmic nucleic acids

NLRP3 is a sensor of host cell damage via DAMPs including reactive oxygen species, potassium ion efflux and more. However, several papers demonstrate that NLRP3 responds to certain nucleic acids in the cytoplasm of cells too. In general, this appears to be mediated by specific adaptor proteins like DHX33 and DDX19A, which contain helicase domains capable of binding to nucleic acids. These adaptors then activate NLRP3. There are some species of nucleic acids such as RNA:DNA hybrids and mitochondrial DNA (mtDNA) that also activate NLRP3, but the adaptor or mechanisms involved remain to be determined.

Figure 3: IFI16 assembles inflammasome in the nucleus, and regulates type I interferon signaling.

A) Recognition of KSHV genomes by IFI16 leads to nuclear IFI16 inflammasome assembly. Once the inflammasome is formed, it translocates to the nucleus where it cleaves pro-IL-18 to its' biologically active form. B) In response to viral genomes or transfected DNA, IFI16 directly associates with DNA motifs through the HIN-200 domains leading to the activation of ER-resident STING. STING subsequently associates with TBK1 leading to the phosphorylation of STING and recruitment and phosphorylation of IRF3. Phosphorylated IRF3 enters the nucleus to trigger the type I interferon gene expression.