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DNA-responsive inflammasomes and their regulators in autoimmunity

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Abstract

Upon sensing microbial and self-derived DNA, DNA sensors initiate innate immune responses. These sensors include the interferon (IFN)-inducible Toll-like receptor 9 (TLR9) and PYHIN proteins. Upon sensing DNA, cytosolic (murine Aim2 and human AIM2) and nuclear (IFI16) PYHIN proteins recruit an adaptor protein (ASC) and pro-caspase-1 to form an inflammasome, which activates caspase-1. The activated caspase-1 cleaves pro-IL-1 β and pro-IL-18 to generate active forms. However, upon sensing cytosolic DNA, the IFI16 protein recruits STING to induce the expression of type I IFN. Recognition of self DNA by innate immune cells contributes to the production of increased levels of type I IFN. Given that the type I IFNs modulate the expression of inflammasome proteins and that the IFN-inducible proteins inhibit the activity of DNA-responsive inflammasomes, an improved understanding of the molecular mechanisms that regulate the activity of DNA-responsive inflammasomes is likely to identify new therapeutic targets to treat autoimmune diseases.

Keywords

Inflammasome; DNA; Interferon; Autoimmunity; Lupus

1. Introduction

Systemic autoimmune diseases include Sjogren's syndrome (SS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and systemic sclerosis (SSc) [1–3]. These diseases are characterized by self antigen-driven immune responses that target host tissues and organs for damage [1, 2]. The main characteristic of these autoimmune diseases is their ability to maintain and amplify immune responses in a feed-forward loop, which ultimately contributes to immuno-pathology [4, 5]. Although, these autoimmune diseases differ with respect to the primary tissues that are targeted by autoantibodies, these diseases share certain common features and mechanisms [2, 5].

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Firstly, autoimmune diseases, including SS, SLE, and RA, exhibit a gender bias [6]. However, the female preponderance varies among these diseases [7]. It has been proposed that factors, such as the X-chromosomal gene dosage effect and sex hormone levels, contribute to gender bias in autoimmune diseases in patients and in certain mouse models [8–10]. Accordingly, the female sex hormone estrogen up-regulates the expression of certain interferon (IFN)-regulating genes (for example, IRF5) and the IFN-inducible genes [10–12]. Additionally, a mutual positive feedback loop between type I IFN and the estrogen receptor- α is likely to contribute to gender bias in autoimmune diseases in mouse models [13].

Secondly, patients with systemic autoimmune diseases exhibit increased serum levels of proinflammatory cytokines [14–16]. These cytokines include tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), and IFNs. Moreover, patients with SS, SLE, and RA exhibit the “IFN-signature” [16, 17]. Notably, it has been reported that mature SLE neutrophils, which are primed by increased levels of type I IFN, die upon exposure to SLE-derived anti-ribonucleoprotein antibodies [18, 19]. Death of neutrophils results in release of neutrophil extracellular traps (NETs). These SLE-associated NETs contain genomic DNA and large amounts of LL37 and HMGB1 proteins, which bind to DNA and potentiate the uptake and recognition of DNA by plasmacytoid DCs (pDCs) [18, 19]. Consequently, the SLE NETs containing self DNA activate innate immune responses, resulting in increased levels of IFN- α and proinflammatory cytokines [18, 19].

Increased levels of proinflammatory cytokines are thought to result from an abnormal activation of innate immune responses that are initiated by innate immune sensors [5, 16]. These innate immune sensors include Toll-like receptors (TLRs), which sense autoantibodies-associated nucleic acids, including DNA [4, 20]. DNA is a potent trigger of inflammatory responses in immune cells [5, 20, 21]. TLR-9, which is localized to the membranous endosomal compartment of B cells and plasmacytoid dendritic cells (pDCs), is a sensor for hypomethylated CpG sequence rich DNA [20]. Moreover, expression of the TLR9 is up-regulated by type I IFN and female sex hormone estrogen in certain cell types [4, 22]. In other cell types (for example, in macrophages), cytosolic DNA activates a potent type I interferon response, which is TLR-independent, but IRF3-dependent [4]. In addition, intracellular DNA in macrophages also induces production of IL-1 β , which suggests an activation of DNA-responsive inflammasome in these cells [23, 24]. Correspondingly, DNA sensors that are localized in the cytoplasm and/or nucleus have been identified in various cell types. These sensors include DNA-dependent activator of IFN-regulatory factors (DAI; also referred to as ZBP1), DExD/H box helicases (DHX9 and DHX36), murine absent in melanoma 2 (Aim2), human AIM2, RNA polymerase III (Pol III), leucine-rich repeat (in Flightless I) interacting protein-1 (Lrrfip1), murine p204, and human IFI16 [21, 24–28]. Upon sensing DNA these sensors initiate innate immune responses, which are not limited to induction of type I IFN: cytosolic DNA also induces maturation of pro-inflammatory cytokines such as IL-1 and IL-18 through formation of inflammasomes, which activates caspase-1 (see below) [24, 29]. Notably, IFN-inducible Aim2 and AIM2 proteins are shown to sense cytosolic DNA to form inflammasomes [27, 30]. The IFN-inducible IFI16 protein can also sense nuclear viral DNA to form the IFI16-ASC inflammasome [28]. However, cytoplasmic IFI16 protein, upon sensing cytosolic DNA (cytosolically administered viral DNA or HSV1 infection), was reported to induce the expression of type I IFN [27].

In this review we discuss the current understanding of the potential role of DNA-responsive inflammasomes and their regulators in the regulation of innate immune responses. Given that aberrant activation of innate immune responses in autoimmune diseases is associated with immunopathology, an improved understanding of the molecular mechanisms through which DNA-responsive inflammasomes and their regulators contribute to immunopathology are likely to present new approaches to alleviate autoantibodies-associated organ damage.

2. Defects in clearance of DNA and autoimmunity

Marginal zone macrophages (MZM) play a critical role in the clearance of apoptotic cells and associated material [3]. MZMs interact with apoptotic material that enters the spleen from circulation [3, 31]. It is known that depletion of MZMs results in increased production of proinflammatory cytokines [3]. In addition, the depletion enhances responsiveness of lymphocytes to apoptotic cell-associated antigens and accelerates the progression of autoimmunity in lupus-prone mice and associated death [31].

The presence of double-stranded DNA (dsDNA or DNA) in cytoplasm due to an infection or damage to tissue is sensed by receptor proteins, which results in the activation of signaling pathways [21, 23]. Similarly, non-self or altered self DNA may be sensed in the nuclear compartment of cells [8]. Studies indicate that lupus-associated pathogenesis includes an abnormal activation of the immune system and an inefficient clearance of dead cellular debris and nucleic acids [3–5]. Consequently, it has been proposed that activation of innate immune responses that are initiated by DNA sensors in cells contributes to inflammatory responses in SLE [3–5]. In SLE, self DNA is often complexed with nuclear antigens and the corresponding autoantibodies [20]. Sensing of this complexed DNA by immune cells with sensors triggers innate immune responses, which lead to the production proinflammatory cytokines, type I IFN, the IFN-signature, and increased production of autoantibodies [Fig. 1]. Thus, the recognition of self DNA by immune cells has a critical role in a feedforward loop, which promotes autoantibody production and associated immunopathology in SLE and possibly other autoimmune diseases [3–5].

SLE is a complex multifactorial disease [1–3]. However, defects in certain DNA-degrading enzymes, which clear potentially pathogenic DNA through degradation, have been linked to the development of lupus-like phenotype in mouse models [32–34]. These proteins include deoxyribonuclease I (DNase I) [32], DNase II [33], and DNase III (also called TREX1) [34]. Accordingly, SLE patients with a non-sense mutation in one allele of DNase I exhibit reduced DNase I activity and increased disease activity [35]. Interestingly, both Toll-like receptor (TLR)-dependent and TLR-independent mechanisms are predicted to be involved in the recognition of the DNase I substrate [4].

In contrast to DNase I, the DNase II is localized in lysosomal compartment of cells, thus, it has access to DNA (derived from apoptotic or necrotic cells) that is engulfed by cells [33]. Notably, mice deficient for DNase II activity are embryonic lethal [33]. This lethality has been attributed to the overproduction of type I IFNs. Accordingly, an additional deficiency of the type I IFN receptor signaling in mice reverses the phenotype that is associated with the DNase II deficiency [33]. Deficiency of cell intrinsic DNase III (or TREX1) in mice results in inflammatory myocarditis and this phenotype can be rescued by genetic ablation of IRF3 or of the type I IFN receptor signaling [34]. Mutations in the *TREX1* gene in humans are associated with SLE-like diseases including familial chilblain lupus and Aicardi-Goutières syndrome [36].

In addition to the above mentioned monogenic form of lupus-like diseases, a recent study involving a family-based approach reported a monogenic form of lupus [37]. The study revealed that a single frame-shift variation in the *DNASE1L3* gene (located on chromosome 3) resulted in a complete lack of DNASE1L3 enzyme activity in individuals. Notably, the disease development in these individuals did not exhibit a gender bias [37], thus, suggesting that the genetic variations in a gene that encodes for a DNA-degrading enzyme can overcome a gender bias in SLE.

3. DNA innate immune sensors in cytoplasm and nucleus

A complex of cytoplasmic proteins, which is termed inflammasome, is activated in response to sensing an infection or DNA [24, 29, 38]. The members of the nucleotide binding and oligomerization domain (NOD)-like receptor (NLR) family proteins can form inflammasomes. These proteins can sense the presence of conserved microbial components in the cytoplasm. The NLR proteins usually share an amino-terminal protein-protein interaction domain such as a CARD or PYD, an intermediary NACHT domain that is required for nucleotide binding and self-oligomerization, and a variable number of carboxy-terminal leucine-rich repeat (LRR) motifs that are involved in sensing pathogen molecules. Upon sensing microbial-derived material, these proteins can directly recruit pro-caspase-1 to form an inflammasome. Notably, it remains unclear whether the NLRs can detect cytosolic DNA to initiate an innate immune response [38, 39]

Cytosolic DNA-sensing inflammasome protein complex contains a DNA sensor protein, an adaptor protein ASC, and pro-caspase-1. The activation of an inflammasome leads to activation of caspase-1, which converts an immature interleukin-1 β and IL-18 to mature secreted form [24, 29, 38, 39]. These secreted proinflammatory cytokines mediate the innate immune responses. As noted above, cytoplasmic DNA triggers a potent inflammatory response [21, 23]. Studies indicate that Aim2, AIM2, and IFI16 proteins, upon sensing DNA, can form an inflammasome [27, 30]. Therefore, these proteins are part of a growing family of DNA-responsive inflammasomes. These proteins share a partially conserved repeat of 200-amino acid residues at the C-terminus (also referred as HIN-200 domain). The HIN-200 domain contains two consecutive OB-folds, which allows these proteins to bind DNA [30]. Additionally, these proteins also contain a homotypic protein-protein interaction pyrin domain (PYD) at the N-terminus. Therefore, these proteins are also referred to as pyrin and HIN-200 domain containing or PYHIN proteins [40]. Interestingly, expression levels and the activity of some of the DNA-responsive inflammasome proteins are negatively regulated by other p200-family proteins (see below) [41, 42].

Studies indicate that increased levels of type I IFN and gender-dependent factors may regulate the activity of inflammasomes [10, 30]. Increased levels of type I IFN suppress the expression of pro-IL-1 β and ASC in certain cell types [42, 43]. However, type I IFNs up-regulate the expression of pro-caspase-1 [43]. Furthermore, sex hormones also regulate the expression of PYHIN proteins and their regulators (see below) [10]. These studies suggest that an imbalance between the activity of DNA-responsive inflammasomes and their negative regulators may contribute to the development of autoimmunity.

3.1 IFI16 as a DNA innate immune sensor

Expression of *IFI16* gene has been noted in a variety of cell types [43–45]. The gene encodes for three isoforms (A, B, and C) of IFI16 protein. These isoforms result due to an alternative splicing of the *IFI16* mRNA. Notably, the B isoform of IFI16 protein is the predominant form in most cell types tested so far [44]. Treatment of IFN-responsive cells with IFN- α , β , or γ induces the expression of *IFI16* gene in a variety of cell types. Expression of IFI16 protein is also induced by activated p53 [46] and treatment of cells with glucocorticoids (dexamethasone) [47], or the male hormone androgen through the androgen receptor [48]. Notably, expression of *IFI16* gene is either reduced or silenced by DNA methylation in certain immortalized human diploid fibroblasts and cancer cell lines [44]. We have noted that the constitutive basal levels of IFI16 protein are not detectable in the primary peripheral blood mononuclear cells (PBMCs) and CD14⁺ monocytes [42]. However, the expression levels are readily detectable in CD19⁺ B lymphocytes. Others have noted that expression of *IFI16* mRNA is detectable in the primary dendritic cells (DCs) [49]. Basal low levels of IFI16 protein are detectable in THP-1 monocytic cell line and type I

IFN-treatment of cells increases the levels significantly [42]. Interestingly, the bulk of the IFN-induced IFI16 protein in THP-1 cells is detected in the cytoplasm [42].

Several lines of evidence implicate a role for IFI16 protein in autoimmunity [44, 50]. Firstly, studies using the primary endothelial cells that overexpress IFI16 protein suggested that the protein is involved in inflammation through regulating the expression of adhesion molecules and chemokines [50]. Accordingly, expression of *IFI16* gene is induced by proinflammatory cytokines in endothelial cells. Moreover, IFI16 protein activates the activity of NF- κ B, a transcription factor that regulates the activity of proinflammatory cytokines [50]. Secondly, a selective blockade of the inhibitory Fc γ receptor (Fc γ RIIB) in human dendritic cells and monocytes induces a type I interferon response program, which results in transcriptional activation of *IFI16* gene [51]. Further, increased levels of IFI16 mRNA have been reported in PBMCs of SLE patients as compared with healthy individuals [52]. However, it remains unclear whether the increases in mRNA levels in PBMCs from SLE patients also result in increases in IFI16 protein levels. Thirdly, IFI16 protein may have a role in DNA-induced activation of human monocyte-derived DCs as well as primary DCs [49].

Interestingly, a study has noted that IFI16 protein can sense intracellular viral DNA [27]. The study showed that, upon sensing cytosolic viral DNA, the IFI16 protein mediated the induction of interferon- β . STING protein, a mediator of the IFN- β induction, was recruited to IFI16 protein after sensing DNA. Moreover, decreasing the expression of IFI16 protein (or its mouse ortholog p204) inhibited the induction of *IFNB* gene and activation of the transcription factors IRF3 and NF- κ B after sensing viral DNA [27]. In contrast to the above study, a study has identified IFI16 protein as a nuclear sensor for viral DNA [28]. The study reported that nuclear replicating Kaposi sarcoma-associated herpesvirus (KSHV)-derived DNA is sensed by IFI16 protein in endothelial cells. Upon sensing the viral nuclear DNA, IFI16 protein recruited the adaptor molecule ASC and procaspase-1 to form a functional IFI16-ASC-procaspase-1 inflammasome. This protein complex was initially detected in the nucleus and subsequently in the perinuclear area of the endothelial cells. Moreover, Caspase-1 activation by KSHV viral DNA was decreased upon silencing of the *IFI16* and *ASC* genes in cells [28]. These studies suggest that IFI16 protein, depending upon the cell type, can sense viral DNA in the cytoplasm as well as in the nucleus to initiate innate immune responses.

3.2 AIM2 inflammasome

Expression of human absent in melanoma 2 (*AIM2*) gene is detectable in a variety of cell types [30]. We recently noted that the constitutive basal levels of the AIM2 protein were not detectable in the PBMCs and CD14⁺ monocytes from healthy donors [42]. However, the expression levels were readily detectable in CD19⁺ B lymphocytes. In addition, basal levels of AIM2 protein were not detectable in THP-1 monocytic cell line [42]. However, treatment of cells with type I IFN or phorbol-12-myristate-13-acetate (PMA), which resulted in differentiation of cells to macrophages, increased the levels of the AIM2 protein significantly [42]. Interestingly, the induced AIM2 protein in cells was detected as two protein bands (~53 and 49 kDa). Currently, it is not clear whether the post-translational modifications of the AIM2 protein result in two protein bands of different sizes. Further, AIM2 protein was detected in both cytoplasm and nucleus [42].

Upon overexpression of AIM2 protein in human cancer cell lines, the protein is detected primarily in the cytoplasm [25, 30]. This overexpressed AIM2 protein can sense cytoplasmic DNA through HIN-200 domain. Upon sensing cytosolic DNA, the AIM2 protein recruits adaptor protein ASC through PYD and ASC protein recruits procaspase-1 through the caspase activation and recruitment domain (CARD) to form an active AIM2 inflammasome. The activated AIM2 inflammasome activates caspase-1, resulting in proteolytic cleavage of

pro-IL-1 β and pro-IL-18 to mature forms for secretion [25, 30]. The AIM2 inflammasome activation in macrophages also induces cell death by pyroptosis [30].

AIM2 protein can also detect cytosolic DNA in human keratinocytes in psoriatic lesions but not in healthy skin [53]. In cultured keratinocytes (KCs), interferon- γ treatment can induce AIM2 expression, and cytosolic DNA triggers the release of IL-1 β via the AIM2 inflammasome in “primed” (primed with IFN- γ and TNF- α) KCs. Moreover, the antimicrobial cathelicidin peptide LL-37, which can interact with dsDNA in psoriatic skin, can neutralize cytosolic DNA in keratinocytes and can block the AIM2 inflammasome activation [53]. Given that cationic peptide LL-37 is found in abundance in SLE patients at sites of inflammation in the skin [53, 54] and kidneys [54] infiltrated by netting neutrophils, which release LL-37 and dsDNA [18, 19], further studies are needed to investigate the role of LL-37 peptide in the regulation of AIM2 inflammasome in tissue and organ-specific inflammation and associated damage.

Based on an earlier study, leukocytes from SLE patients express increased levels of AIM2 mRNA [52]. However, no correlation between AIM2 expression and SLE disease activity was evident [52]. Interestingly, a recent study has noted that expression of *AIM2* gene is induced by IFN- α in endothelial progenitor cells (EPCs) and circulating angiogenic cells (CACs) [56]. Given that the incidence of premature atherosclerosis increases up to a 50-fold in SLE patients and the incidence is associated with endothelial dysfunction (an accelerated cell death of endothelial cells by apoptosis) [56], it has been proposed that an imbalance between endothelial cell damage and repair that is triggered by increased inflammasome activity in SLE patients may contribute to an accelerated atherosclerosis through potentiation of IL-18 activation [56, 57]. Therefore, further studies are needed to investigate the potential role of the AIM2 inflammasome in endothelial damage and associated increased risk to develop atherosclerosis in SLE patients.

3.3 Aim2 inflammasome

Expression of murine *Aim2* gene is detectable in splenic B (B220⁺), T (CD3⁺), and bone marrow-derived macrophages (BMDMs; CD11b⁺) from non lupus-prone C57BL/6 mice [41, 58, 59]. However, steady-state levels of the Aim2 protein were appreciably higher in the splenic B cells than in T cells or BMDMs [58]. Treatment of BMDMs, but not splenic T or B cells, by IFN- α induced the expression of *Aim2* gene [59]. Moreover, treatment of androgen receptor (AR) positive cells with the male hormone androgen also increased the steady-state levels of Aim2 mRNA and protein [59]. Further, activation of Toll-like receptors (TLR3, 4, and 9) signaling in murine macrophage cell lines (RAW264.7 and J774.A1) and BMDMs also stimulated the expression of the *Aim2* gene [59].

Generation of *Aim2*-deficient mice on the mixed (129 $\times$ C57BL/6) [60, 61] and C57BL/6 [26] genetic background has revealed that the *Aim2*-deficiency in mice increases their susceptibility to certain bacterial and viral infections in the presence of increased serum levels of type I IFN (IFN- β). Correspondingly, the immune cells (BMDMs and splenic cells) from the *Aim2*-knockout mice were unable to activate caspase-1 (and pro-IL-1 β and pro-IL-18) upon infection by certain intracellular pathogens [26, 60, 61]. Thus, indicating that the activity of the Aim2 inflammasome is important for providing protection against certain infections. Additionally, a study has noted that *Francisella* infection triggers activation of the Aim2 inflammasome in murine dendritic cells [62]. Of note, studies have revealed that the Aim2 protein is not required for the production of type I IFN in mice when mice are infected with certain pathogens [26, 60]. Moreover, Aim2 protein suppresses the type I IFN expression and the expression of certain IFN-regulated genes in mice (see below) [41, 58]. In addition, studies have revealed that lupus-prone NZB and B6.*Nba2* female mice express reduced levels of the Aim2 mRNA and protein in immune cells (including splenic B and T

cells) as compared to age and gender-matched C57BL/6 mice [41]. Of importance, reduced levels of the Aim2 protein in lupus-prone female mice are associated with reduced basal levels of activated caspase-1 (p20) in immune cells [41]. Reduced expression of Aim2 protein and decreased activation of caspase-1 in lupus-prone female mice (as compared to age-matched C57BL/6 female mice) is consistent with an increased expression of type I IFN and certain IFN-regulated genes in these mice [41].

4. Regulators of DNA-responsive inflammasomes

The activity of certain inflammasomes is negatively regulated by type I IFNs in part through suppression of pro-IL-1 β expression [43]. Interestingly, the suppression was reported to be STAT1-dependent, thus, raising the possibility that the IFN-inducible proteins mediate the anti-inflammatory activities of the type I IFNs. Consistent with the above prediction, the activity of the Aim2 [41, 63] and AIM2 [42] inflammasomes are negatively regulated by the IFN-inducible p202 and IFI16 proteins, respectively.

4.1 p202 protein as a negative regulator of the Aim2 inflammasome

p202 protein (encoded by the *Ifi202* gene) is a member of the IFN-inducible p200-family of proteins [64, 65]. In addition to type I IFNs, the expression of the *Ifi202* gene is induced by IL-6 [66] and the female hormone estrogen [12]. In contrast, the expression is suppressed by activated p53 [67], growth factors [68], and E2F1 [69]. The p202 protein lacks homotypic protein-protein interaction PYD in the N-terminus [30]. Moreover, the protein is detected in cytoplasm as well as in the nucleus and the activation of the IFN-signaling in cells potentiates the nuclear localization [70, 71]. The nuclear localization of p202 protein may be important for its ability to modulate the transcriptional activity of several immunomodulatory transcription factors, including the NF- κ B, c-Jun/AP-1, and c-Myc [64, 65, 72].

Several lines of evidence support a role for the p202 protein in murine lupus [65, 72]. Generation of the B6.*Nba2* congenic (congenic for the New Zealand Black-derived *Nba2* interval on the C57BL/6 genetic background) mice and gene expression analysis identified the IFN-inducible *Ifi202* gene as a candidate lupus susceptibility gene [73]. The study also revealed that increased expression of p202 protein in splenic B cells is associated with increased production of autoantibodies and defects in apoptosis of B cells. Interestingly, type I IFN-signaling is required for the development of lupus-like disease in the B6.*Nba2* congenic mice and the lack of disease development in the type-I IFN-signaling deficient mice is associated with reduced expression of the *Ifi202* gene [74]. Given that the activation of IFN-signaling in cells potentiates the nuclear localization of the p202 protein [70, 71], it was not surprising that a sub-congenic line (the B6.*Nba2*-C) of the B6.*Nba2* mice, which harbored the *Ifi202* gene (and other *Ifi200*-family genes) and did not produce type I IFN (thus inhibiting the nuclear localization of p202 protein), failed to produce autoantibodies [75, 76]. Notably, the study revealed that *Ifi202* gene is not sufficient to induce a lupus-like disease in the B6.*Nba2*-C mice. Further, the study provided evidence for epistatic interactions between the *Ifi202* gene and other *Nba2* genes (including the *Fcgr2b* gene) [75]. Correspondingly, the generation of *Aim2* deficient mice on the mixed (129 x B6) genetic background indicated that *Aim2*-deficiency induces the expression of type I IFN and *Ifi202* gene [41]. In addition, increased levels of p202 protein in the *Aim2*-deficient cells suppressed the expression of the inhibitory receptor Fc γ RIIB [58], reduced expression of which on mixed genetic background is associated with the development of a lupus-like disease [77]. Thus, the above genetic studies indicate that epistatic interactions involving the *Nba2* genes, including the *Aim2*, *Ifi202* and *Fcgr2b*, contribute to increased production of autoantibodies and type I IFN in the B6.*Nba2* female mice and possibly other strains of mice.

Interestingly, recent studies have provided novel insights into a role for the p202 protein in lupus diseases through the regulation of the Aim2 inflammasome activity. Firstly, knockdown of *Ifi202* expression in BMDMs resulted in increased activation of caspase-1 in response to cytosolic DNA [63]. Secondly, increased expression of p202 protein in a macrophage cell line resulted in increased production of type I IFN and activation of IFN-signaling, which reduced steady-state levels of the Aim2 and FcγRIIB proteins [58]. Thirdly, increased expression of p202 protein in immune cells from lupus-prone female mice is associated with reduced steady-state levels of pro-IL-1β and Aim2 protein [41]. Given that the p202 protein can heterodimerize with other members of the p200-proteins [64, 65], including the Aim2 protein, it is likely that increased levels of p202 protein in immune cells inhibit the activity of Aim2 inflammasome in part through binding to the Aim2 protein.

4.2 IFI16 protein as a negative regulator of the AIM2 inflammasome

As noted above, the IFN-inducible IFI16 protein contains both PYD and HIN-200 domains [44]. Interestingly, subcellular localization (cytoplasmic *versus* nuclear) of endogenous IFI16 protein appears to depend on cell type [78]. Nuclear IFI16 protein is reported to interact with a number of transcriptional modulatory proteins, including the p53, E2F1, BRCA1, pRb, hTERT, and AIM2 [42, 44]. Therefore, two recent studies that identified IFI16 protein as an innate immune sensor for cytosolic DNA [27] as well as nuclear DNA [28] to induce different innate immune responses (induction of type I IFN *versus* IL-1β production) raised the possibility that IFI16 protein could regulate the activity of the AIM2 inflammasome through binding to AIM2 protein and down-regulating the expression of AIM2 and other inflammasome proteins. Correspondingly, the study involving young, old, and senescent human diploid fibroblasts (HDFs) indicated that expression of IFI16 and AIM2 proteins is inversely correlated [79]. In addition, increased type I IFN expression and activation of IFN-signaling in old HDFs was associated with increased levels of IFI16 protein and reduced levels of AIM2 protein. In contrast, the senescence-associated secretory phenotype in HDFs, which results in IL-1β production, was associated with increased levels of AIM2 protein and reduced levels of IFI16 protein. Further, overexpression of IFI16 protein in transfected cells inhibited the activation of caspase-1 by the AIM2-ASC inflammasome [42]. Accordingly, knockdown of IFI16 expression in THP-1 monocytic cells increased the basal levels of pro-IL-1β and activated caspase-1. Notably, the knockdown of IFI16 levels also increased the synthetic DNA [poly (dA:dT)]-induced activation of caspase-1 by the AIM2 inflammasome [42]. Thus, these studies support the idea that increased levels of the IFI16 protein in certain cell types inhibit the activity of AIM2 inflammasome through multiple mechanisms.

5. Conclusions

Anti-inflammatory glucocorticoids are widely used to treat patients with autoimmune diseases. However, recognition of self nucleic acids, including DNA, by innate immune cells and induction of innate immune responses remain a major challenge in treating patients. In particular, release of self DNA by SLE NETs, which is sensed by innate immune cells through TLR9 and other DNA sensors, results in antinuclear autoantibodies and the production of higher levels of type I IFN through a feedforward loop. Given that increased levels of type I IFN also regulate the expression of DNA sensors and the activity of DNA-responsive inflammasomes, an improved understanding of the molecular mechanisms through which innate immune cells sense self DNA and initiate innate immune responses is likely to identify new therapeutic targets to treat autoimmune diseases effectively. Therefore, it is important to understand the role of DNA-responsive inflammasomes and their negative regulators in autoimmune diseases.

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Highlights

- DNA-responsive inflammasomes contribute to the development of autoimmunity.
- The interferon-inducible PYHIN proteins sense cytosolic and nuclear DNA.
- The PYHIN proteins initiate different innate immune responses after sensing DNA.
- The AIM2 inflammasomes is negatively regulated by IFI16 protein.
- Activation of inflammasomes may contribute to end-organ disease in SLE.

Figure 1. Sensing of self DNA by DNA sensor proteins and induction of innate immune responses by cell of the innate immune system in mouse models of SLE.

Figure 2.
Upon sensing DNA, AIM2 and IFI16 proteins activate different innate immune responses.

Table 1

Murine and human cytosolic and nuclear DNA sensors and their associated innate immune responses upon sensing DNA.

DNA sensor	Ligand(s) tested	Cell type tested	Subcellular location	Signaling molecules	Innate immune response	Ref.
Murine p202	Synthetic and calf thymus DNA	BMM [*] , RAW264.7, J774.A1	Cytoplasm	Not known	Inhibition of Aim2 inflammasome activation	[63]
Murine Aim2	Synthetic, Viral, and bacterial DNA	BMDM TEMs HEK293T	Cytoplasm	ASC and pro-caspase-1	Aim2 inflammasome activation and production of IL-1 β and IL-18	[26, 60, 61, 62]
Human AIM2	Synthetic DNA, vaccinia DNA	293T, HEK293 THP-1 Macrophage	Cytoplasm	ASC and pro-caspase-1	AIM2 inflammasome activation and production of IL-1 β and IL-18	[25, 30]
IFI16	VACV, Synthetic DNA	BMDMs THP-1, HEK293 Endothelial cells	Cytoplasm	STING, TBK1, IRF3, and NF- κ B	Type I interferon production	[27]
IFI16	KSHV DNA		Nucleus	ASC and pro-caspase-1	IFI16 inflammasome activation and production of IL-1 β and IL-18	[28]
Murine p204	VACV (70mer), Synthetic DNA	RAW264.7B MDM	cytoplasm	Not known	Type I IFN production	[27]

* BMM, Bone marrow macrophage; BMDM, Bone marrow-derived macrophage, KSHV, Kaposi's sarcoma-associated virus; TEM, thioglycollate elicited macrophage; VACV, Vaccinia virus.