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Innate Sensing of DNA Virus Genomes

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Abstract

DNA viruses are linked to many infectious diseases and contribute significantly to human morbidity and mortality worldwide. Moreover, DNA viral infections are usually life long and hard to eradicate. Under certain circumstances, these viruses can cause fatal disease, especially in children and immunocompromised patients. An efficient innate immune response against these viruses is critical, not only as the first line of host defense against viral infection but also for mounting more specific and robust adaptive immunity against the virus. Recognition of the viral DNA genome is the very first step of this whole process and is crucial for understanding viral pathogenesis as well as for preventing and treating DNA virus-associated diseases. This review focuses on the current state of our knowledge on how human DNA viruses are sensed by the host innate immune system and how viral proteins counteract this immune response.

1. SIGNIFICANCE

DNA viral infections are a major health concern worldwide. Acute infection with DNA viruses can lead to life-threatening diseases, while chronic infection results in the persistence of the virus through manipulation of host immune responses. Many DNA viruses establish a persistent and/or latent infection in the host, which increases the potential for developing severe disease during the lifetime of the host. The most-studied DNA viruses that are involved in innate immunity are double-stranded DNA (dsDNA) viruses. Members of this group of viruses possess DNA genomes in either a circular or linear form. Although some of the larger DNA viruses encode their own DNA polymerases, the smaller DNA viruses depend on polymerases of host cells for genome replication.

Many members of this group of viruses are characterized as etiological agents of human disease, including cancer. For example, some human papillomavirus (HPV) strains are highly oncogenic and can lead to cervical cancer and head and neck cancers (1). Adenoviruses cause cold-like symptoms in humans and have also been intensively studied as oncolytic viral vectors since they stimulate both innate and adaptive immune responses, which are needed for antitumor therapy (2). Chronic hepatitis B virus (HBV) infection leads to liver cancer, which is the fifth leading cause of cancer death among men (3). Infection with herpes simplex virus (HSV)-1 or HSV-2 not only leads to painful blisters or ulcers at the site of infection (mouth or genitals, respectively) but also can lead to more serious outcomes, including encephalitis (4). Varicella-zoster virus (VZV) infection is present in more than 90% of adults in the United States. Primary infection with VZV leads to acute

varicella, also known as chicken pox. Reactivation of VZV often lead to herpes zoster, also known as shingles (5). Human cytomegalovirus (HCMV) causes serious symptoms in immunocompromised patients. Congenital HCMV infection can lead to defective development of brain, liver, spleen, and lung and to stunted growth (6). Epstein-Barr virus (EBV) is etiologically associated with various type of cancers, including Burkitt lymphoma, Hodgkin lymphoma, post-transplant lymphomas, gastric cancers, and almost all endemic nasopharyngeal carcinomas (7). Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi's sarcoma, primary effusion lymphoma, multicentric Castleman's disease, and, most recently, KSHV inflammatory cytokine syndrome. The incidence of Kaposi's sarcoma is much higher in immunosuppressed individuals, such as patients who are undergoing transplantation or chemotherapy or are infected with human immunodeficiency virus (HIV). In fact, Kaposi's sarcoma is the most common neoplasm in patients with AIDS in the United States and globally (8). The two most famous poxviruses are variola virus, the etiological agent of smallpox, and vaccinia virus (VACV), which was used as a vaccine against smallpox (9). Given the diverse pathologies associated with DNA viruses, a thorough understanding of immune and inflammatory responses against their components is essential in deciphering and combatting DNA viral infections. In addition, such knowledge will benefit the development of oncolytic viruses and safer virus-based vectors used in gene therapy.

2. DETECTION OF DNA VIRUSES BY PATTERN RECOGNITION RECEPTORS

The innate immune system serves as the first line of host defense by deploying a number of germline-encoded receptors called pattern-recognition receptors (PRRs) to detect pathogen infection. PRRs recognize conserved molecular structures that are essential for the life cycle of pathogens; these are known as pathogen-associated molecular patterns (PAMPs) (10). Several PRR families have been identified so far, including toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), and the cytosolic DNA sensors. Here we focus exclusively on sensors involved in DNA recognition, which are summarized in Figure 1.

2.1. TLR DNA Sensors

To date, 10 TLRs have been identified in humans, and among them, TLR9 is the only known DNA sensor. TLR9 specifically recognizes unmethylated CpG motifs in dsDNA (CpG DNA), which are commonly found in bacterial and viral genomes but are rare in mammalian genomes (11, 12). TLR9 is sequestered in the endoplasmic reticulum (ER) in unstimulated cells and rapidly traffics to endolysosomes after CpG DNA stimulation (13). The endosomal TLR9 recruits the adaptor protein MyD88, then recruits TRAF6 and the IKK complex, leading to activation of NF- κ B and eventually to the induction of inflammatory cytokines (14). TLR9 is expressed in plasmacytoid dendritic cells and has the capacity to induce type I interferon (IFN) production in response to DNA viruses including HPV, EBV, KSHV, and Merkel cell polyomavirus (15–18).

Although TLR9 is the only TLR that recognizes DNA, other TLR family members also play a role in detecting DNA virus infections. For example, EBV-encoded RNAs, which may be released from infected cells in exosomes, also activate TLR7 to promote IFN α production (19). Likewise, KSHV infection activates TLR3 in primary monocytes, probably through recognition of viral RNA transcripts packaged in the tegument, leading to the induction of IFNs (20). TLR2 was identified as a host factor that detects the HCMV glycoproteins gB and gH and initiates inflammatory cytokine secretion in response to HCMV infection (21). In addition, TLR2 has been reported to be responsible for MCP-1 induction upon EBV infection (22). TLR4 has been shown to be activated upon HCMV infection in monocytes and induces IFN β and TNF α (23).

2.2. RLR DNA Sensors

The RIG-I–MAVS pathway has been well established to play a pivotal role in sensing cytosolic RNA, especially 5'-triphosphate RNA, which is generated during RNA virus infection (24). Although RIG-I predominantly recognizes RNA virus infection, the RIG-I–MAVS pathway is also activated during infection with several DNA viruses, including HSV-1, EBV, and KSHV (25–27). Furthermore, RNA polymerase III (RNA Pol III) is able to recognize AT-rich dsDNA, such as poly(dA:dT), and induce IFN β production in a manner dependent on the RIG-I–MAVS pathway (28, 29). The underlying mechanism is that RNA Pol III transcribes AT-rich dsDNA into dsRNA containing a 5'-triphosphate moiety, which is a known ligand of RIG-I (28, 29). Inhibition of RNA Pol III expression or its enzymatic activity significantly impairs IFN induction against several DNA viruses, including adenovirus, HSV-1, and EBV (28, 29). Thus, recognition of cytoplasmic AT-rich dsDNA through the RNA Pol III–RIG-I–MAVS axis represents a possible cross talk between innate sensing of RNA viruses and DNA viruses.

2.3. STING, cGAS, IFI16, and Other DNA Sensors

In a screen to identify new components of innate immunity, overexpression of a protein named stimulator of IFN genes (STING; also known as MPYS, MITA, or ERIS) was found to potently induce IFN β promoter-driven luciferase activity (30–32). Subsequent studies demonstrated that STING is essential for type I IFN induction in response to cytosolic DNA as well as HSV-1 infection, and STING-deficient mice were extremely susceptible to HSV-1 infection (33). Although STING is able to bind DNA directly (34), the physiological relevance of this interaction is still unclear. Instead, STING exerts its essential role in the DNA sensing pathway mainly as an adaptor protein to activate TBK1–IRF3 and NF- κ B, leading to the subsequent transcription of antiviral genes. In the presence of cytosolic DNA, STING traffics from the ER to an ER-Golgi intermediate compartment and then to the Golgi apparatus and eventually relocates to perinuclear regions, where it is degraded, possibly in the lysosomal compartment (35, 36). The underlying mechanism of the initiation and regulation of STING trafficking is still poorly understood, although lysine 63-linked ubiquitination of STING and several autophagy-related proteins has been proposed to play a role in this process (35–38). During trafficking, STING recruits and activates the kinase TBK1, which in turn phosphorylates IRF3, leading to IRF3-mediated type I IFN induction (39, 40). Many DNA viruses were reported to activate the STING pathway, including HSV-1, adenovirus, VACV, HPV, and KSHV (41). STING was also found to directly

recognize cyclic dinucleotides (CDNs)—small molecules secreted by some bacteria to function as second messengers—and induce a type I IFN response (42).

Since the discovery of STING, several putative DNA sensors have been reported to detect cytosolic DNA and activate type I IFN production dependent on STING. IFN γ -inducible protein 16 (IFI16) was the first reported PRR that detects cytosolic DNA and activates antiviral gene expression via STING. It was identified in a DNA affinity pull-down screen as binding a 70-bp dsDNA fragment derived from the VACV genome (43). In the presence of intracellular DNA, IFI16 interacts with STING to induce TBK1-dependent IFN β responses. Knockdown of IFI16 or its mouse ortholog p204 by small interfering RNA significantly inhibited gene induction in response to DNA stimulation or HSV-1 infection (43, 44). IFI16 is predominantly a nuclear protein, and a number of recent studies have reported that IFI16 also recognizes viral genomes in the nucleus and activates inflammasome-mediated immune responses, which are discussed below.

In a small interfering RNA screen with 59 members of the DExD/H family of helicases, DEAD-box helicase 41 (DDX41) was found to be required for type I IFN induction in myeloid dendritic cells and THP-1 cells stimulated with dsDNA or infected with HSV-1 (45). DDX41 was shown to directly bind various DNAs and then recruit STING and TBK1 to activate downstream signal transduction. Another study from the same group reported that DDX41 also bound bacterial CDNs with a higher affinity than that of STING and then induced type I IFN production via STING, TBK1, and IRF3 (46). Although these data suggest that DDX41 plays a role parallel to STING's in recognizing cytoplasmic DNA and CDNs, other groups found that knockdown of DDX41 had little effect on IFN β induction in response to DNA stimulation or DNA virus infection (34, 47). Therefore, further studies using DDX41-knockout animals or cell lines will be necessary to clarify the role of DDX41 in the DNA sensing pathway.

Several proteins previously known to be involved in the DNA damage response have also been implicated as cytosolic DNA sensors. DNA-dependent protein kinase (DNA-PK) is a heterotrimeric protein complex consisting of three subunits: Ku70, Ku80, and the catalytic subunit DNA-PKcs. All three components of the DNA-PK complex were pulled down using biotinylated DNA in HEK 293T cells (48). Further studies have shown that upon DNA stimulation or VACV infection, DNA-PK-mediated cytokine induction was dependent on STING, TBK1, and IRF3 (48). Ku70 was also found to interact with the human T lymphotropic virus type 1 reverse transcription intermediate ssDNA90 and to promote ssDNA90-triggered innate immune responses in a STING-dependent manner (49). Another DNA damage sensor, meiotic recombination 11 homolog A (Mre11), has also been reported to be a cytosolic DNA sensor that activates the STING pathway (50). Interestingly, Mre11 was found to be required for type I IFN induction in response to dsDNA stimulation but not to HSV-1 infection (50).

Although the proteins mentioned above all seem to play roles in detecting cytosolic DNA, their contributions to DNA-mediated induction of type I IFNs and cytokines are either partial or cell type specific. In 2013, the discovery of cyclic-GMP-AMP (cGAMP) synthase (cGAS) as a novel DNA sensor further advanced our understanding of the signaling

mechanisms employed by innate DNA sensing (47). Unlike other STING-related sensors, cGAS does not bind STING directly. Instead, it catalyzes the production of cGAMP from ATP and GTP after binding DNA, and cGAMP then interacts with a STING dimer on the ER membrane (47, 51, 52). Upon binding cGAMP or bacterial CDNs in the central crevice, the STING dimer undergoes an extensive conformational change that is postulated to release its C-terminal tail (53, 54), which has been reported to be required for TBK1 recruitment and activation (40). cGAS has been shown to recognize DNA from a large range of species, including DNA viruses (HSV-1, KSHV, VACV, adenovirus, etc.), retroviruses (HIV-1, HIV-2, etc.), bacteria, parasites, and fungi, as well as self-DNA leaked from the nucleus and mitochondria (reviewed in 55). Subsequent studies using cells from cGAS-deficient mice showed a complete loss of type I IFN production in response to DNA stimulation or DNA virus infection (HSV-1, VACV, and murine gammaherpesvirus 68), indicating a nonredundant role for cGAS in DNA-mediated type I IFN responses (56, 57). Moreover, cGAMP generated by cGAS in DNA virus-infected cells can be transferred to uninfected neighboring cells through gap junctions, where it promotes STING activation and antiviral immunity independently of type I IFN signaling (58). This finding provides a novel host strategy that serves to rapidly convey antiviral immunity from virally infected cells to bystander cells. Interestingly, the Beclin-1 protein, involved in autophagy, has been shown to interact with cGAS and regulate innate immune responses, indicating functional cross talk between the innate immunity and autophagy pathways (59, 60).

2.4. Inflammasome DNA Sensors

The inflammasome is a large, multiprotein complex that has crucial roles in the immune response to viral infection (61). Absent in melanoma 2 (AIM2), a member of the PYHIN protein family, was identified as a receptor for cytosolic DNA that activates the inflammasome pathway (62–65). AIM2 contains an N-terminal PYRIN domain and one C-terminal HIN200 domain. After binding DNA via its HIN200 domain, AIM2 associates with the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) through a homotypic PYRIN:PYRIN interaction. ASC further recruits and activates caspase-1 through a homotypic CARD:CARD interaction. Caspase-1 in turn causes proteolytic maturation of the proinflammatory cytokines interleukin (IL)1 β and IL18. Knockdown of AIM2 abrogated caspase-1 activation in response to cytoplasmic dsDNA and VACV infection, while reconstitution of AIM2 in nonresponsive HEK 293T cells conferred responsiveness to cytoplasmic DNA (62, 63). More importantly, analysis of AIM2-deficient mice further confirmed the role of AIM2 in inflammasome activation induced by cytosolic DNA and DNA virus (VACV and mouse cytomegalovirus) in vivo (66, 67).

IFI16 is another member of the PYHIN protein family that was first characterized as a DNA sensor for the type I IFN response (see discussion above). However, unlike other PYHIN family proteins, which have exclusive nuclear or cytoplasmic localization patterns, the IFI16 protein localizes predominantly in the nucleus but can also shuttle between the cytosol and nucleus in various cell types (68). Several recent studies have reported that IFI16 also recognizes DNA viral genomes in the nucleus, where episomal genomes of the large DNA viruses reside and replicate. Similar to sensing by AIM2, the nuclear sensing of viral DNA by IFI16 activates the inflammasome pathway through ASC and caspase-1, leading to IL1 β

and IL18 production. The inflammasome-associated viral DNAs recognized by IFI16 in the nucleus are from herpesviruses, including HSV-1, HCMV, KSHV, and EBV (69). Moreover, IFI16 is also able to detect single-stranded DNA (ssDNA) derived from HIV-1 proviral DNA to invoke caspase-1 activation and pyroptosis (70, 71).

Many NLR inflammasomes are also implicated in sensing DNA virus infection. For example, NLRP3 has been shown to be responsible for IL1 β production upon challenge with modified vaccinia virus Ankara, VZV, or DNA from adenovirus (72–74). The NLRP1 inflammasome was activated in primary monocytes upon KSHV infection (75). Interestingly, another NLR protein, NLRX1, acts negatively on DNA sensing to reduce IFN induction in response to KSHV reactivation (76).

2.5. Other DNA Sensors

DNA-dependent activator of IRFs (DAI; also known as ZBP1) was the first reported sensor to recognize dsDNA in the cytoplasm and activate type I IFNs through NF- κ B and IRF3 (77). DAI was also found to play a role in IFN β production in fibroblasts induced by HSV-1 or HCMV infection (77, 78). However, DAI-knockout mice demonstrated normal DNA-mediated cytokine production (79). The DExD/H-box helicases DHX36 and DHX9 are two other proposed DNA sensors, which were found to bind CpG-A DNA [oligodeoxyribonucleotide (ODN) 2216] and CpG-B DNA (ODN 2006), respectively, and activate a MyD88-dependent pathway in plasmacytoid dendritic cells (80). Suppression of DHX9 or DHX36 significantly reduced the cytokine responses of plasmacytoid dendritic cells to a DNA virus (HSV) but not to an RNA virus (influenza A virus), suggesting a DNA sensing specificity (80). However, the fact that TLR9 is indispensable for CpG DNA-induced immune responses suggests that DHX36 and DHX9 might be required for TLR9 pathway activation, rather than acting as direct DNA sensors.

3. DNA SENSING SPECIFICITY

Discriminating host and viral DNA is very important for the host to mount appropriate innate responses against viral infection. In most circumstances, precise sensing of viral DNA requires the recognition of multiple factors, such as localization, length, sequence, modification, availability, and structure of host and viral DNA. On the other hand, many features of the DNA sensors themselves are also important, such as localization, DNA binding affinity, and crystal structure. In the following sections, we describe some examples of DNA sensors and how DNA sensing specificity is determined.

3.1. TLR9-Dependent DNA Sensing

The specificity of TLR9-dependent DNA sensing is largely reliant upon the location and sequence of target DNA. TLR9 is recruited to the late endosome and lysosome only when DNA stimuli are present in these compartments. Notably, ectopic localization of a chimeric TLR9 to the cell surface led to the recognition of host genomic DNA, indicating that localization of TLR9 is critical for discrimination between host and virus DNAs (81, 82). TLR9 predominately binds to CpG DNA, which is rarely present in host DNA. Further characterization has indicated that in addition to a CG dinucleotide, the position, number,

surrounding nucleotides, and secondary structure of the ODNs are all critical for optimal TLR9 signaling (reviewed in 83). The minimal ODNs required for optimal TLR9 activation in human peripheral blood mononuclear cells were defined by the sequence TCG[T]₆–10CG[T]_{9–19} and a minimum length of 21 nt (84). Most strikingly, it was recently reported that although ODNs shorter than 21 nt cannot activate TLR9 by themselves, short, CpG-containing ODNs (sODNs) as short as 2 nt can facilitate TLR9 activation. Experiments have indicated that when mixed with sODNs, even mammalian genomic DNA can successfully mount TLR9-dependent innate immune responses (85). This provides an important clue on how short DNA degradation products help to activate TLR9 quickly upon DNA virus infection, particularly when TLR9 engages very low concentrations of DNA viral genomes.

Other than the classic CpG sequence, recent studies showed that TLR9 can also recognize RNA:DNA hybrids without CpG sequences (86). A synthetic 60mer GU-repetitive RNA strand and 60mer AC-repetitive DNA strand hybrid (R:D60) was shown to induce TLR9-dependent proinflammatory cytokine and antiviral type I IFN production in dendritic cells. Notably, the AC-repetitive ssDNA has no TLR9 activation potential, suggesting that R:D60 serves as the actual TLR9 agonist.

3.2. cGAS-Dependent DNA Sensing

The DNA binding specificity of cGAS has been studied extensively. Multiple groups have reported that cGAS recognizes host or viral dsDNA in a sequence-independent but length-dependent manner. Experiments determined that cGAS binds to a minimum of 40 bp in human myeloid cells and 25 bp in mouse myeloid cells. Binding is largely reliant on its DNA binding domain, located in the C terminus. This finding was based on crystal structural analysis from multiple independent groups (56, 87–89). Evidence has shown that defects in either Trex1 or DNaseII, enzymes responsible for DNA degradation in the cytoplasm or lysosome, respectively, lead to accumulation of cGAMP and elevated IFN-stimulated gene expression in mice, indicating that host DNAs in the cytosol successfully activate cGAS-STING signaling (90–92). Therefore, the DNA binding specificity of cGAS seems to rely mostly on DNA availability in the cytoplasm rather than on differences between host and viral DNA.

Sequence dependency of cGAS recognition was recently studied. ODNs containing TTAGGG motifs were shown to suppress cGAS activation in human monocytes. A recent study found that A151, an ODN, binds to cGAS in a sequence- and backbone-specific manner and that it competes with stimulatory DNA for binding to cGAS, resulting in the attenuation of cGAS-STING signaling (93). More importantly, ODN A151 was previously characterized as a therapeutic agent against inflammation-driven oncogenesis, collagen-induced arthritis, and uveitis (reviewed in 94). This study provides a mechanistic explanation of immunosuppressive oligonucleotides, which could advance the development of therapeutic agents against cGAS-STING-related autoimmune diseases. A recent study reported that cGAS could also recognize a unique form of nucleic acid, RNA:DNA hybrids, which successfully triggered production of cGAMP (95).

3.3. AIM2- and IFI16-Dependent DNA Sensing

The DNA binding activities of AIM2 and IFI16 are determined by HIN domains present in both molecules. The binding is achieved by electrostatic attraction between the positively charged HIN domain residues and the dsDNA sugar-phosphate backbone, in a DNA sequence-independent manner (96). Similar to cGAS, AIM2 and IFI16 also recognize DNA in a length-dependent manner. The DNA sugar-phosphate backbone binds to the HIN domain of AIM2 and IFI16, which limits the size of DNA for optimal signaling in human myeloid cells to approximately 80 bp (96). A model has been proposed in which the PYRIN domain binds to HIN under a resting state to keep inflammasome signaling checked. Upon DNA engagement, the PYRIN domain dissociates from the HIN domain and binds to ASC for downstream signaling.

Another follow-up study revealed more details about the two HIN domains (HINa and HINb) upon DNA sensing. Interestingly, X-ray crystal structure studies have indicated that while HINb contacts both strands of DNA, HINa specifically contacts only one strand of DNA, which is consistent with previous findings that HINa binds to ssDNA and dsDNA with comparable affinity (97–99). In addition to their different DNA binding preferences, HINb is important for inducing DNA-triggered IFN β production, while HINa inhibits IFN β production. However, the distinct roles in DNA sensing played by these two domains may suggest tight regulation following signal transduction.

Regarding sequence and structure binding specificity, a recent study reported that special structures in DNA, such as quadruplex structures, can preferentially bind to IFI16 and trigger more potent type I IFN responses than those produced by the same sequence in dsDNA. Such structures are intrinsic in many viral genomes, such as those of EBV and HPV (100, 101), as well as in some GC-rich host genome elements such as telomeres and promoter regions (102). Although no evidence suggests that IFI16 can distinguish host DNA from viral DNA, this study extends our understanding that IFI16 can selectively sense specific structures and mount different innate immune responses accordingly. Interestingly, ODN A151 has also been shown to directly bind to AIM2 and prevent the recruitment of ASC and the subsequent assembly of the inflammasome complex (103). Thus, the TTAGGG motifs contained in A151 might bind to multiple DNA sensors, and its broad inhibitory effect could indicate that TTAGGG serves as a conserved immunosuppressive motif in humans and mice. Notably, these TTAGGG motifs widely exist in telomerase sequences, suggesting a potential self-protection strategy for the human genome.

3.4. Other DNA Sensors

The specificity of other DNA sensors in the DNA sensing process is relatively uncharacterized. For example, although the crystal structure of DNA-PK has been reported by multiple groups, the specificity of its DNA binding activity remains largely unknown (104). DDX41 is reported to bind dsDNA and c-di-GMP directly by a DEAD domain (46, 105), but the crystalized DEAD domain could not bind with dsDNA and c-di-GMP in vitro (106). RNA Pol III recognizes a specific form of DNA featuring repetitive dA:dT and induces type I IFNs through the RIG-I pathway, although RNA Pol III has not yet been

shown to bind viral DNA sequences (28, 29). Studies of other DNA sensors are needed to better understand the intrinsic nature of the DNA sensing machinery.

4. MECHANISMS OF DNA SENSING

Viral genomes govern virus survival and replication. Therefore, the packaging of a virus must be strong enough to protect the genome from environmental and immunological challenges. The life cycle of a DNA virus can be summarized in the following six steps: attachment, penetration, intracellular transport, replication, assembly, and release. In this section, we discuss current findings on how and when viral DNA is engaged by DNA sensing pathways within the host.

4.1. TLR Receptors (Viral Attachment and Penetration)

TLRs are located either in the plasma membrane or in endosomes and lysosomes. Specifically, TLR1, TLR2, TLR4, TLR5, and TLR6 are located primarily in the plasma membrane, where they sense the danger signal when pathogens bind to cells. Attachment is the first step that initiates the process of viral infection, which relies on the recognition of viral capsid proteins or glycoproteins on the viral envelope by specific receptors on the host cell surface. Upon binding, signaling pathways are triggered, which results in either direct penetration or fusion of virions to endosomes/vesicles. The most well-characterized plasma membrane-localized receptors that are responsible for recognizing viral infection and triggering innate immune responses are the TLRs. TLR2 is the predominant cell surface receptor involved in antiviral defenses, paired with coreceptors such as TLR1 and TLR6. TLR2 is expressed by antigen-presenting cells, such as monocytes, macrophages, dendritic cells, B cells, and T cells. It is also found in some nonimmune cells, such as mucosal epithelial cells and keratinocytes (109, 110). TLR2 has been shown to play a critical role upon human or mouse CMV infection in vitro and in vivo. Consistent with this, defects in TLR2 function are associated with HCMV infection in liver transplant patients (111). In addition, evidence has shown that TLR2 plays a role in immune responses against other DNA viruses, such as HSV, EBV, VZV, and VACV (reviewed in 41, 112). The recognition of PAMPs from these DNA viruses, however, involves not their DNA genome but rather glycoproteins on the viral surface. For example, TLR2 and TLR1 form a heterodimer that triggers NF- κ B responses upon recognition of the HCMV glycoproteins gB and gH (21). TLR4 has also been shown to facilitate the control of VACV infection in mice. Following VACV infection, mice lacking TLR4 exhibited greater mortality compared to wild-type mice, without impaired production of proinflammatory cytokines in the lungs (113).

Upon binding to the cell surface, most commonly, enveloped viruses undergo receptor-dependent endocytosis, by which virions are delivered to endosomes or other intracellular organelles (114). Viruses strive to escape from endocytosis through various strategies. This involves fusion of the viral envelope with endosomal membranes for enveloped viruses and forming pores or lysing the endosomal membrane by employing viral peptides for nonenveloped viruses (115). Viruses that fail to escape end up in late endosomes and lysosomes for degradation. In these compartments, viruses undergo disassembly, potentially exposing the genome to TLRs localized there, such as TLR3, TLR7, TLR8, and TLR9.

Several independent groups have demonstrated that TLR9 is translocated to late endosomes or lysosomes, where it detects CpG motifs contained in viral DNA (normally unmethylated, compared to methylated motifs in host DNA) (11, 117). TLR9 localization is important for optimal DNA sensing for the following reasons. First, the acidic milieu in endosomes is critical for viral disassembly (118). Second, endosomal proteases such as cathepsins and asparagine endopeptidases are required for optimal TLR9 signaling, as the N-terminal ectodomain of TLR9 needs to be processed to recruit MyD88 (119–122). Third, endosomal localization may also provide a mechanism to minimize recognition of host DNAs that are in the nucleus. Evidence shows that surface-localized mutant TLR9 can respond to vertebrate genomic DNA and cause systemic lethal inflammation in transgenic TLR9 mutant mice (81, 82).

4.2. Cytosolic DNA Sensors (Viral Intracellular Transportation)

Following endocytosis, most DNA viruses travel through the cytoplasm before they reach the nucleus. During this process, DNA genomes are protected by the viral capsid, which is not disassembled until the viral DNA genome is released into the nucleus. Therefore, questions arise about how exactly viral DNA is engaged by cytosolic DNA sensors under physiological conditions. It is easier to understand how some DNA viruses, such as smallpox virus, trigger a STING-dependent signaling pathway since they replicate in the cytoplasm (123). Moreover, DNA viruses such as the polyomavirus simian virus 40 disassemble their capsid in the ER and release their viral DNA into the cytoplasm, which could also potentially trigger the cytosolic DNA sensing pathway (124). However, in the case of many other viruses, such as herpesviruses, the viral DNA is exposed only in the nucleus. One possible explanation for cytosolic sensing is that herpesviral DNAs from defective virion particles can leak into the cytoplasm and are then sensed by cytosolic DNA sensors. Evidence for this includes the fact that the HSV-1 and HCMV capsids can be ubiquitinated and subjected to proteasome degradation in macrophages, leading to the release of viral DNA into the cytoplasm, where it is sensed by IFI16 (44). Another explanation is that herpesvirus infections lead to cellular stress-dependent leaking of mitochondrial DNA (mtDNA) into the cytoplasm, which activates the cGAS-STING pathway and type I IFN production (126). Others also reported that mtDNA released from mitochondria can trigger innate immunity through the cGAS-STING pathway (127, 128). mtDNA also activates NLRP3-dependent IL1 β production, suggesting that mtDNA can serve as a damage-associated molecular pattern to trigger innate immune responses (129). It is quite possible that viral infection stresses the cell and leads to the detection of both mtDNA and viral genomes, either sequentially or concomitantly. How these two processes are linked remains to be elucidated.

4.3. Nuclear DNA Sensors (Viral DNA Replication)

Most DNA viruses release their genomic DNA in the nucleus of the cell, which makes nuclear DNA sensors critical for viral detection. IFI16 is so far the best-characterized nuclear DNA sensor; it triggers both type I IFN responses and inflammasome responses. It is interesting that IFI16 is localized both in the nucleus and in the cytoplasm and has been shown to participate in antiviral responses (69). Similarly, Ku70 was reported to be localized in the nucleus and then to translocate to the cytoplasm upon DNA binding to form a

complex with cytoplasmic STING (130). These findings provide an additional explanation for how cytosolic DNA sensors engage viral DNA. Moreover, cGAS has been shown to be present in the nucleus in certain cell types and therefore may play a role in nuclear sensing of viral DNA (131, 132).

4.4. DNA Sensing During Viral Assembly and Release

Virions of DNA viruses replicating in the nucleus are often assembled in close proximity to DNA replication centers. In most of these processes, viral DNA is protected from immune detection by capsids and/or envelopes. Few studies have been focused on detection of DNA viruses specifically during assembly or release. However, sensing mechanisms similar to those described above might occur in these processes, depending on the cellular compartment where these events happen.

5. VIRAL REGULATION OF DNA SENSING PATHWAYS

Appropriate innate immune responses stimulated by viral DNA are important for maintaining host integrity and homeostasis. Insufficient innate immunity often results in ineffective clearance of viral infection, which leads to manifestation of disease symptoms. In this section, our aim is to summarize regulators of DNA sensing pathways by viruses and the mechanisms underlying the battle between virus and host (Table 1). Due to space restrictions, we limit our summary to viral inhibitors targeting either DNA sensors or adaptor proteins that recruit DNA sensors upon stimulation.

5.1. Viral Proteins Sequester DNA Sensors from Viral DNA Genomes

Binding viral DNA is the initial step of the innate immune response. Therefore, many viruses block this sensing step for immune evasion. One good example is the HCMV-encoded pUL83 protein; pUL83 binds IFI16 and affects its oligomerization, which is important for DNA sensing (133–135). In addition, another HCMV protein, pUL97, hijacks IFI16 and traps it within the cytoplasm, away from the HCMV genome in the nucleus. Interestingly, IFI16 was found in newly assembled HCMV virions (136). This is similar to the observation that STING was found in virions from HSV-1-infected cells, which facilitate transfer of STING to newly infected cells (137). This could be one host strategy that takes advantage of the virus to initiate antiviral signaling against viral infection. KSHV ORF52, which targets cGAS, binds to both DNA and cGAS and effectively blocks the engagement of DNA with cGAS, thereby inhibiting cGAS activation (138). Notably, the ORF52 proteins encoded by other gammaherpesviruses, such as EBV and murine gammaherpesvirus 68, also exhibit similar phenotypes by the same mechanism, suggesting a conserved strategy shared among gammaherpesviruses (41, 138). The VACV C16 protein has been shown to bind to the Ku70-Ku80 complex and block DNA sensing by DNA-PK in fibroblasts (139). Viral proteins that directly target other innate DNA sensors are still under investigation.

5.2. Viral Proteins Disrupt Innate Immune Signaling from Adaptors

Upon sensing viral DNA, a signaling complex based on innate immune adaptor proteins is quickly assembled for fast and optimal immune activation. Therefore, disrupting an adaptor's connection with other molecules is one classic strategy employed by DNA viruses.

A46R encoded by VACV was reported to act as an inhibitor of TLR9 signaling by interacting with MyD88 (140). STING, as the key adaptor of many of the innate DNA sensors identified to date, is targeted by multiple viral proteins. KSHV vIRF1 binds to both STING and TBK1, thereby effectively blocking the STING-TBK1 interaction, a critical step for type I IFN production (141). Similarly, other viral proteins that target STING include but are not limited to HPV E7 and adenovirus E1A (summarized in Table 1). Additionally, many other viral proteins have also been shown to inhibit signaling from inflammasome complexes. Although it is currently unclear whether these inflammasomes are activated by DNA or other virion stimuli, many DNA viruses have been shown to inhibit inflammasome signaling (143). For example, myxoma poxvirus encodes M13L protein, which contains a PYRIN domain that interacts with ASC and negatively regulates inflammasome formation by preventing the activation of IL1 β and IL18 (143a, 144). Another viral protein, KSHV ORF63, inhibits the activation of NLRP1 and NLRP3 inflammasomes to prevent the secretion of active IL1 β and IL18 (75).

5.3. Viral Proteins Regulate Posttranslational Modifications

Phosphorylation, ubiquitination, and other posttranslational modifications are important for pathway regulation, which can be targeted by viral inhibitors. For example, lysine 63–linked polyubiquitination of STING has been reported to be disrupted during viral infection by multiple viral proteins, including HBV polymerase (145). Moreover, phosphorylation of STING was also shown to be blocked in the presence of KSHV vIRF1 (141), and KSHV ORF64, a deubiquitinase, was shown to suppress RIG-I-mediated activation and IFN signaling by reducing RIG-I ubiquitination (146). The fact that multiple viruses possess the ability to affect posttranslational modification of innate immune sensors also underlines the importance of posttranslational modifications in DNA sensing pathways.

5.4. Viral Proteins Inducing Protein Degradation

Some viral proteins have the ability to facilitate immune sensor degradation and thereby block IFN responses. For example, HSV-1 ICP0 has been shown to promote the degradation of IFI16 in a proteasome-dependent manner and thus to block IFI16-dependent signaling (147). However, evidence from two other groups indicates that ICP0 alone is neither sufficient nor required for IFI16 degradation (148, 149). A possible explanation for these findings might be that ICP0 is one of many HSV-1 viral proteins that induce IFI16 degradation, and hence there is a certain level of redundancy in the system. Interestingly, another group reported that, instead of causing degradation, ICP0, ICP4, and US3-PK stabilize STING in HEp-2 cells, and this stabilization is required for optimal HSV-1 replication (150).

6. IMPORTANCE OF DNA SENSING PATHWAYS IN THERAPEUTICS

Considering the central role of DNA pathways in the innate immune response against DNA viruses, it may be feasible to harness this response for cures to infectious diseases and cancers. For example, treatment with CpG ODN, a TLR9 agonist, induced intrahepatic myeloid cell aggregates that enabled a massive expansion of the cytotoxic T lymphocyte population locally in the liver. The antiviral immune response eradicated HBV-infected

hepatocytes during chronic infection and led to viral clearance (151). Another group reported that intraperitoneal administration of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a small molecule that activates mouse STING, significantly induced IFNs and cytokine production and reduced HBV DNA replication intermediates in the livers of HBV-injected mice (152).

The interaction between innate immune responses and cancer had been recognized some time ago, and the potential of employing the innate immune response for cancer immunotherapy has also been widely studied (153). TLR9 agonists have been shown to inhibit tumor activity as single agents and to enhance the development of antitumor T cell responses when used as therapeutic vaccine adjuvants (154). On the other hand, recent studies on the cGAS-STING pathway demonstrated its crucial role in type I IFN-dependent, CD8 α^+ dendritic cell-mediated antitumor T cell responses as well as radiation-induced tumor cell death (155, 156). Intratumoral administration of STING agonists, including cGAMP, bacterial CDNs, and other derivatives, has shown dramatic antitumor effects in mouse models of skin, colon, brain, pancreatic, breast, and B cell malignancies (reviewed in 157). STING agonists can also serve as adjuvants to enhance the antitumor effect of various cancer therapies, such as vaccines, radiation therapy, and PD-L1 immune checkpoint blockade (157). Further studies and clinical trials using these agonists may lead to the development of human-specific therapies in the near future.

7. SENSING OF RETROVIRUSES

In addition to DNA viruses, RNA viruses—in particular retroviruses—have also been shown to activate DNA sensing pathways. Although these viruses do not possess a DNA genome, they use reverse transcriptase to convert their RNA genome into DNA, which has been shown to trigger DNA sensing pathways (158). HIV-1 is the most well-studied retrovirus. HIV infection can lead to AIDS and can render patients susceptible to several types of cancer, including non-Hodgkin lymphoma, Kaposi's sarcoma, and cervical cancer (159).

7.1. DNA Sensors of Retroviruses

Studies have shown that HIV-1 and other retroviruses can activate cGAS-dependent innate immunity by stimulating the synthesis of cGAMP (158). In addition, IFI16 is also able to detect ssDNA derived from HIV-1 proviral DNA to invoke caspase-1 activation and pyroptosis (70, 71). The mechanism for how HIV-1 DNA is recognized by DNA sensors has been recently studied. It has been reported that primary HIV-1 ssDNA during early infection of macrophages is localized in the cytoplasm and activates cGAS-dependent type I IFNs (160). Notably, this mechanism might apply to other retroviruses, which undergo reverse transcription in the cytoplasm.

7.2. Specificity of Retroviral DNA Sensing

A recent study revealed that HIV reverse transcription in the cytoplasm generates a stem-loop DNA structure and that this short Y-form DNA activates cGAS in a sequence-dependent manner. Specifically, the presence of unpaired guanosines in short single-stranded 3'- and 5'-flanking sequences is critical for recognition of Y-form DNA and activates cGAS

signaling. Furthermore, within this structure, even 12–20 nt of Y-form DNA can be highly IFN stimulatory in a cGAS-dependent manner in peripheral blood mononuclear cells (160). Notably, most studies utilize viral dsDNA as a stimulatory agent for the DNA sensing pathway, while cytosolic sensors for viral ssDNA are rarely reported. Evidence has shown that STING can bind to transfected ssDNA in cells or in vitro and is responsible for ssDNA-triggered type I IFN production (30, 33, 34). However, further evidence of STING binding to viral ssDNA during bona fide viral infection is lacking.

In addition to the sequence-specific recognition of HIV-1 DNA, studies have shown that RNA:DNA hybrids, a viral nucleic acid structure typically formed during reverse transcription, can be recognized by multiple DNA sensors (161). As described in Section 3, two independent groups reported that synthetic RNA:DNA hybrids have the potential to activate TLR9- or cGAS-dependent pathways (86, 95). Thus, it remains to be explored whether retroviral RNA:DNA hybrids indeed serve as TLR9/cGAS agonists during the course of a viral infection.

8. CONCLUDING REMARKS AND PERSPECTIVES

A long list of DNA sensors has been assembled since the first discovery of mammalian TLR9 in 2000 (162, 163). Numerous studies in the past decade have established the central role of DNA sensing pathways in initiating innate immune responses against DNA virus infection. Tremendous progress has been made in unraveling the molecular mechanism of how viral DNAs are recognized by DNA sensors and how the signaling pathways are activated and elaborately regulated. In parallel, our understanding of how DNA viruses evade or even take advantage of innate immunity to facilitate their replication has also been greatly expanded. Despite this recent progress, many interesting questions remain to be answered. A key question is, why are there so many DNA sensors? Is there physiological redundancy or cell specificity between these different DNA sensing pathways? The genomes of most DNA viruses reside and replicate in the nucleus. Hence, two additional emerging questions are, how do cytosolic DNA sensors gain access to these viral DNAs, and how does nuclear sensing of viral genomes, such as by IFI16, propagate the signal to STING in the cytosol? Furthermore, many RNA viruses and DNA viruses antagonize both host RNA and DNA sensing pathways, and the exact mechanisms need to be elucidated. For example, in addition to its central role in DNA sensing, STING is also known to inhibit RNA virus replication. This suggests that significant cross talk likely occurs between RNA and DNA nucleic acid sensing pathways, a phenomenon that remains poorly understood.

Targeting of DNA sensing pathways has proved to be a potential therapeutic approach to treat infection-induced acute and chronic diseases such as hepatitis B and liver cirrhosis as well as cancers in animal models. However, the key challenge now is to translate these findings into the development of new vaccines and antiviral drugs for clinical use in humans. Emerging evidence indicates that DNA sensing pathways also play critical roles in antitumor immune responses as well as the development of several autoimmune diseases. Future efforts in this fast-moving field will further shed light on the role of DNA sensors in viral infection and inflammatory diseases as well as in the treatment of infections and cancer.

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LITERATURE CITED

- Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. 2015 Epidemiology of human papillomavirus-positive head and neck squamous cell carcinoma. *J. Clin. Oncol* 33:3235–42 [PubMed: 26351338]
- Niemann J, Kuhnel F. 2017 Oncolytic viruses: adenoviruses. *Virus Genes* 53:700–6 [PubMed: 28702840]
- Masrouj-Roudsari J, Ebrahimpour S. 2017 Causal role of infectious agents in cancer: an overview. *Caspian J. Intern. Med* 8:153–58 [PubMed: 28932365]
- Roizman B, Knipe DM, Whitley RJ. 2013 Herpes simplex viruses In *Fields Virology*, ed. Knipe DM, Howley PM, pp. 1823–97. Philadelphia: Lippincott Williams & Wilkins 6th ed.
- Gershon AA, Gershon MD. 2013 Pathogenesis and current approaches to control of varicella-zoster virus infections. *Clin. Microbiol. Rev* 26:728–43 [PubMed: 24092852]
- Griffiths P, Baraniak I, Reeves M. 2015 The pathogenesis of human cytomegalovirus. *J. Pathol* 235:288–97 [PubMed: 25205255]
- Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. 2015 The immunology of Epstein-Barr virus-induced disease. *Annu. Rev. Immunol* 33:787–821 [PubMed: 25706097]
- Dittmer DP, Damania B. 2016 Kaposi sarcoma-associated herpesvirus: immunobiology, oncogenesis, and therapy. *J. Clin. Investig* 126:3165–75 [PubMed: 27584730]
- Chan WM, McFadden G. 2014 Oncolytic poxviruses. *Annu. Rev. Virol* 1:119–41 [PubMed: 25839047]
- Wu J, Chen ZJ. 2014 Innate immune sensing and signaling of cytosolic nucleic acids. *Annu. Rev. Immunol* 32:461–88 [PubMed: 24655297]
- Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, et al. 2004 TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat. Immunol* 5:190–98 [PubMed: 14716310]
- Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, et al. 2000 A Toll-like receptor recognizes bacterial DNA. *Nature* 408:740–45 [PubMed: 11130078]
- Kim YM, Brinkmann MM, Paquet ME, Ploegh HL. 2008 UNC93B1 delivers nucleotide-sensing toll-like receptors to endolysosomes. *Nature* 452:234–38 [PubMed: 18305481]
- Kawai T, Akira S. 2011 Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34:637–50 [PubMed: 21616434]
- Hasan UA, Bates E, Takeshita F, Biliato A, Accardi R, et al. 2007 TLR9 expression and function is abolished by the cervical cancer-associated human papillomavirus type 16. *J. Immunol* 178:3186–97 [PubMed: 17312167]
- Fiola S, Gosselin D, Takada K, Gosselin J. 2010 TLR9 contributes to the recognition of EBV by primary monocytes and plasmacytoid dendritic cells. *J. Immunol* 185:3620–31 [PubMed: 20713890]
- West JA, Gregory SM, Sivaraman V, Su L, Damania B. 2011 Activation of plasmacytoid dendritic cells by Kaposi's sarcoma-associated herpesvirus. *J. Virol* 85:895–904 [PubMed: 20980519]
- Shahzad N, Shuda M, Gheit T, Kwun HJ, Cornet I, et al. 2013 The T antigen locus of Merkel cell polyomavirus downregulates human Toll-like receptor 9 expression. *J. Virol* 87:13009–19 [PubMed: 24067965]

19. Quan TE, Roman RM, Rudenga BJ, Holers VM, Craft JE. 2010 Epstein-Barr virus promotes interferon- α production by plasmacytoid dendritic cells. *Arthritis Rheum* 62:1693–701 [PubMed: 20178121]
20. West J, Damania B. 2008 Upregulation of the TLR3 pathway by Kaposi's sarcoma-associated herpesvirus during primary infection. *J. Virol* 82:5440–49 [PubMed: 18367536]
21. Boehme KW, Guerrero M, Compton T. 2006 Human cytomegalovirus envelope glycoproteins B and H are necessary for TLR2 activation in permissive cells. *J. Immunol* 177:7094–102 [PubMed: 17082626]
22. Gaudreault E, Fiola S, Olivier M, Gosselin J. 2007 Epstein-Barr virus induces MCP-1 secretion by human monocytes via TLR2. *J. Virol* 81:8016–24 [PubMed: 17522215]
23. Yew KH, Carpenter C, Duncan RS, Harrison CJ. 2012 Human cytomegalovirus induces TLR4 signaling components in monocytes altering TIRAP, TRAM and downstream interferon-beta and TNF-alpha expression. *PLOS ONE* 7:e44500 [PubMed: 22970235]
24. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, et al. 2004 The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat. Immunol* 5:730–38 [PubMed: 15208624]
25. West JA, Wicks M, Gregory SM, Chugh P, Jacobs SR, et al. 2014 An important role for mitochondrial antiviral signaling protein in the Kaposi's sarcoma-associated herpesvirus life cycle. *J. Virol* 88:5778–87 [PubMed: 24623417]
26. Rasmussen SB, Jensen SB, Nielsen C, Quartin E, Kato H, et al. 2009 Herpes simplex virus infection is sensed by both Toll-like receptors and retinoic acid-inducible gene-like receptors, which synergize to induce type I interferon production. *J. Gen. Virol* 90:74–78 [PubMed: 19088275]
27. Samanta M, Iwakiri D, Kanda T, Imaizumi T, Takada K. 2006 EB virus-encoded RNAs are recognized by RIG-I and activate signaling to induce type I IFN. *EMBO J* 25:4207–14 [PubMed: 16946700]
28. Ablasser A, Bauernfeind F, Hartmann G, Latz E, Fitzgerald KA, Hornung V. 2009 RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat. Immunol* 10:1065–72 [PubMed: 19609254]
29. Chiu YH, MacMillan JB, Chen ZJ. 2009 RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell* 138:576–91 [PubMed: 19631370]
30. Ishikawa H, Barber GN. 2008 STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* 455:674–78 [PubMed: 18724357]
31. Zhong B, Yang Y, Li S, Wang YY, Li Y, et al. 2008 The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity* 29:538–50 [PubMed: 18818105]
32. Sun W, Li Y, Chen L, Chen H, You F, et al. 2009 ERIS, an endoplasmic reticulum IFN stimulator, activates innate immune signaling through dimerization. *PNAS* 106:8653–58 [PubMed: 19433799]
33. Ishikawa H, Ma Z, Barber GN. 2009 STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* 461:788–92 [PubMed: 19776740]
34. Abe T, Harashima A, Xia T, Konno H, Konno K, et al. 2013 STING recognition of cytoplasmic DNA instigates cellular defense. *Mol. Cell* 50:5–15 [PubMed: 23478444]
35. Barber GN. 2015 STING: infection, inflammation and cancer. *Nat. Rev. Immunol* 15:760–70 [PubMed: 26603901]
36. Konno H, Konno K, Barber GN. 2013 Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. *Cell* 155:688–98 [PubMed: 24119841]
37. Ni G, Konno H, Barber GN. 2017 Ubiquitination of STING at lysine 224 controls IRF3 activation. *Sci. Immunol* 2:eaah7119 [PubMed: 28763789]
38. Saitoh T, Fujita N, Hayashi T, Takahara K, Satoh T, et al. 2009 Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. *PNAS* 106:20842–46 [PubMed: 19926846]
39. Liu S, Cai X, Wu J, Cong Q, Chen X, et al. 2015 Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. *Science* 347:aaa2630 [PubMed: 25636800]

40. Tanaka Y, Chen ZJ. 2012 STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. *Sci. Signal* 5:ra20 [PubMed: 22394562]
41. Ma Z, Damania B. 2016 The cGAS-STING defense pathway and its counteraction by viruses. *Cell Host Microbe* 19:150–58 [PubMed: 26867174]
42. Burdette DL, Monroe KM, Sotelo-Troha K, Iwig JS, Eckert B, et al. 2011 STING is a direct innate immune sensor of cyclic di-GMP. *Nature* 478:515–18 [PubMed: 21947006]
43. Unterholzner L, Keating SE, Baran M, Horan KA, Jensen SB, et al. 2010 IFI16 is an innate immune sensor for intracellular DNA. *Nat. Immunol* 11:997–1004 [PubMed: 20890285]
44. Horan KA, Hansen K, Jakobsen MR, Holm CK, Sørensen S, et al. 2013 Proteasomal degradation of herpes simplex virus capsids in macrophages releases DNA to the cytosol for recognition by DNA sensors. *J. Immunol* 190:2311–19 [PubMed: 23345332]
45. Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu YJ. 2011 The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat. Immunol* 12:959–65 [PubMed: 21892174]
46. Parvatiyar K, Zhang Z, Teles RM, Ouyang S, Jiang Y, et al. 2012 The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and cyclic di-AMP to activate a type I interferon immune response. *Nat. Immunol* 13:1155–61 [PubMed: 23142775]
47. Sun L, Wu J, Du F, Chen X, Chen ZJ. 2013 Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science* 339:786–91 [PubMed: 23258413]
48. Ferguson BJ, Mansur DS, Peters NE, Ren H, Smith GL. 2012 DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. *eLife* 1:e00047 [PubMed: 23251783]
49. Wang J, Kang L, Song D, Liu L, Yang S, et al. 2017 Ku70 senses HTLV-1 DNA and modulates HTLV-1 replication. *J. Immunol* 199:2475–82 [PubMed: 28821586]
50. Kondo T, Kobayashi J, Saitoh T, Maruyama K, Ishii KJ, et al. 2013 DNA damage sensor MRE11 recognizes cytosolic double-stranded DNA and induces type I interferon by regulating STING trafficking. *PNAS* 110:2969–74 [PubMed: 23388631]
51. Wu J, Sun L, Chen X, Du F, Shi H, et al. 2013 Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science* 339:826–30 [PubMed: 23258412]
52. Ouyang S, Song X, Wang Y, Ru H, Shaw N, et al. 2012 Structural analysis of the STING adaptor protein reveals a hydrophobic dimer interface and mode of cyclic di-GMP binding. *Immunity* 36:1073–86 [PubMed: 22579474]
53. Gao P, Ascano M, Zillinger T, Wang W, Dai P, et al. 2013 Structure-function analysis of STING activation by c[G(2',5')pA(3',5')p] and targeting by antiviral DMXAA. *Cell* 154:748–62 [PubMed: 23910378]
54. Zhang X, Shi H, Wu J, Zhang X, Sun L, et al. 2013 Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. *Mol. Cell* 51:226–35 [PubMed: 23747010]
55. Chen Q, Sun L, Chen ZJ. 2016 Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. *Nat. Immunol* 17:1142–49 [PubMed: 27648547]
56. Li XD, Wu J, Gao D, Wang H, Sun L, Chen ZJ. 2013 Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects. *Science* 341:1390–94 [PubMed: 23989956]
57. Schoggins JW, MacDuff DA, Imanaka N, Gainey MD, Shrestha B, et al. 2014 Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. *Nature* 505:691–95 [PubMed: 24284630]
58. Ablasser A, Schmid-Burgk JL, Hemmerling I, Horvath GL, Schmidt T, et al. 2013 Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP. *Nature* 503:530–34 [PubMed: 24077100]
59. Liang Q, Seo GJ, Choi YJ, Ge J, Rodgers MA, et al. 2014 Autophagy side of MB21D1/cGAS DNA sensor. *Autophagy* 10:1146–47 [PubMed: 24879161]
60. Liang Q, Seo GJ, Choi YJ, Kwak MJ, Ge J, et al. 2014 Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. *Cell Host Microbe* 15:228–38 [PubMed: 24528868]

61. Kanneganti TD. 2010 Central roles of NLRs and inflammasomes in viral infection. *Nat. Rev. Immunol* 10:688–98 [PubMed: 20847744]
62. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. 2009 AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* 458:509–13 [PubMed: 19158676]
63. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, et al. 2009 AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 458:514–18 [PubMed: 19158675]
64. Bürckstümmer T, Baumann C, Blüml S, Dixit E, Dürnberger G, et al. 2009 An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat. Immunol* 10:266–72 [PubMed: 19158679]
65. Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, et al. 2009 HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Science* 323:1057–60 [PubMed: 19131592]
66. Rathinam VA, Jiang Z, Waggoner SN, Sharma S, Cole LE, et al. 2010 The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol* 11:395–402 [PubMed: 20351692]
67. Fernandes-Alnemri T, Yu JW, Juliana C, Solorzano L, Kang S, et al. 2010 The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. *Nat. Immunol* 11:385–93 [PubMed: 20351693]
68. Dell'Oste V, Gatti D, Giorgio AG, Gariglio M, Landolfo S, De Andrea M. 2015 The interferon-inducible DNA-sensor protein IFI16: a key player in the antiviral response. *New Microbiol* 38:5–20 [PubMed: 25742143]
69. Diner BA, Lum KK, Cristea IM. 2015 The emerging role of nuclear viral DNA sensors. *J. Biol. Chem* 290:26412–21 [PubMed: 26354430]
70. Monroe KM, Yang Z, Johnson JR, Geng X, Doitsh G, et al. 2014 IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV. *Science* 343:428–32 [PubMed: 24356113]
71. Jakobsen MR, Bak RO, Andersen A, Berg RK, Jensen SB, et al. 2013 IFI16 senses DNA forms of the lentiviral replication cycle and controls HIV-1 replication. *PNAS* 110:E4571–80 [PubMed: 24154727]
72. Delaloye J, Roger T, Steiner-Tardivel QG, Le Roy D, Knaup Reymond M, et al. 2009 Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome. *PLOS Pathog* 5:e1000480 [PubMed: 19543380]
73. Muruve DA, Petrilli V, Zaiss AK, White LR, Clark SA, et al. 2008 The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* 452:103–7 [PubMed: 18288107]
74. Nour AM, Reichelt M, Ku CC, Ho MY, Heineman TC, Arvin AM. 2011 Varicella-zoster virus infection triggers formation of an interleukin-1 β (IL-1 β)-processing inflammasome complex. *J. Biol. Chem* 286:17921–33 [PubMed: 21385879]
75. Gregory SM, Davis BK, West JA, Taxman DJ, Matsuzawa S, et al. 2011 Discovery of a viral NLR homolog that inhibits the inflammasome. *Science* 331:330–34 [PubMed: 21252346]
76. Ma Z, Hopcraft SE, Yang F, Petrucelli A, Guo H, et al. 2017 NLRX1 negatively modulates type I IFN to facilitate KSHV reactivation from latency. *PLOS Pathog* 13:e1006350 [PubMed: 28459883]
77. Takaoka A, Wang Z, Choi MK, Yanai H, Negishi H, et al. 2007 DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature* 448:501–5 [PubMed: 17618271]
78. DeFilippis VR, Alvarado D, Sali T, Rothenburg S, Früh K. 2010 Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1. *J. Virol* 84:585–98 [PubMed: 19846511]
79. Ishii KJ, Kawagoe T, Koyama S, Matsui K, Kumar H, et al. 2008 TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. *Nature* 451:725–29 [PubMed: 18256672]
80. Kim T, Pazhoor S, Bao M, Zhang Z, Hanabuchi S, et al. 2010 Aspartate-glutamate-alanine-histidine box motif (DEAH)/RNA helicase A helicases sense microbial DNA in human plasmacytoid dendritic cells. *PNAS* 107:15181–86 [PubMed: 20696886]

81. Barton GM, Kagan JC, Medzhitov R. 2006 Intracellular localization of Toll-like receptor 9 prevents recognition of self DNA but facilitates access to viral DNA. *Nat. Immunol* 7:49–56 [PubMed: 16341217]
82. Mouchess ML, Arpaia N, Souza G, Barbalat R, Ewald SE, et al. 2011 Transmembrane mutations in Toll-like receptor 9 bypass the requirement for ectodomain proteolysis and induce fatal inflammation. *Immunity* 35:721–32 [PubMed: 22078797]
83. Rathinam VA, Fitzgerald KA. 2011 Innate immune sensing of DNA viruses. *Virology* 411:153–62 [PubMed: 21334037]
84. Pohar J, Kuznik Krajnik A, Jerala R, Bencina M. 2015 Minimal sequence requirements for oligodeoxyribonucleotides activating human TLR9. *J. Immunol* 194:3901–8 [PubMed: 25780037]
85. Pohar J, Lainscek D, Ivicak-Kocjan K, Cajnko MM, Jerala R, Bencina M. 2017 Short single-stranded DNA degradation products augment the activation of Toll-like receptor 9. *Nat. Commun* 8:15363 [PubMed: 28530246]
86. Rigby RE, Webb LM, Mackenzie KJ, Li Y, Leitch A, et al. 2014 RNA:DNA hybrids are a novel molecular pattern sensed by TLR9. *EMBO J* 33:542–58 [PubMed: 24514026]
87. Civril F, Deimling T, de Oliveira Mann CC, Ablasser A, Moldt M, et al. 2013 Structural mechanism of cytosolic DNA sensing by cGAS. *Nature* 498:332–37 [PubMed: 23722159]
88. Kato K, Ishii R, Goto E, Ishitani R, Tokunaga F, Nureki O. 2013 Structural and functional analyses of DNA-sensing and immune activation by human cGAS. *PLOS ONE* 8:e76983 [PubMed: 24116191]
89. Kranzusch PJ, Lee AS, Berger JM, Doudna JA. 2013 Structure of human cGAS reveals a conserved family of second-messenger enzymes in innate immunity. *Cell Rep* 3:1362–68 [PubMed: 23707061]
90. Gao D, Li T, Li XD, Chen X, Li QZ, et al. 2015 Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. *PNAS* 112:E5699–705 [PubMed: 26371324]
91. Ablasser A, Hemmerling I, Schmid-Burgk JL, Behrendt R, Roers A, Hornung V. 2014 TREX1 deficiency triggers cell-autonomous immunity in a cGAS-dependent manner. *J. Immunol* 192:5993–97 [PubMed: 24813208]
92. Ahn J, Ruiz P, Barber GN. 2014 Intrinsic self-DNA triggers inflammatory disease dependent on STING. *J. Immunol* 193:4634–42 [PubMed: 25261479]
93. Steinhagen F, Zillinger T, Peukert K, Fox M, Thudium M, et al. 2018 Suppressive oligodeoxynucleotides containing TTAGGG motifs inhibit cGAS activation in human monocytes. *Eur. J. Immunol* 48:605–11 [PubMed: 29215161]
94. Bayik D, Gursel I, Klinman DM. 2016 Structure, mechanism and therapeutic utility of immunosuppressive oligonucleotides. *Pharmacol. Res* 105:216–25 [PubMed: 26779666]
95. Mankan AK, Schmidt T, Chauhan D, Goldeck M, Honing K, et al. 2014 Cytosolic RNA:DNA hybrids activate the cGAS-STING axis. *EMBO J* 33:2937–46 [PubMed: 25425575]
96. Jin T, Perry A, Jiang J, Smith P, Curry JA, et al. 2012 Structures of the HIN domain:DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor. *Immunity* 36:561–71 [PubMed: 22483801]
97. Ni X, Ru H, Ma F, Zhao L, Shaw N, et al. 2016 New insights into the structural basis of DNA recognition by HINa and HINb domains of IFI16. *J. Mol. Cell Biol* 8:51–61 [PubMed: 26246511]
98. Yan H, Dalal K, Hon BK, Youkharibache P, Lau D, Pio F. 2008 RPA nucleic acid-binding properties of IFI16-HIN200. *Biochim. Biophys. Acta* 1784:1087–97 [PubMed: 18472023]
99. Brazda V, Coufal J, Liao JC, Arrowsmith CH. 2012 Preferential binding of IFI16 protein to cruciform structure and superhelical DNA. *Biochem. Biophys. Res. Commun* 422:716–20 [PubMed: 22618232]
100. Murat P, Zhong J, Lekieffre L, Cowieson NP, Clancy JL, et al. 2014 G-quadruplexes regulate Epstein-Barr virus-encoded nuclear antigen 1 mRNA translation. *Nat. Chem. Biol* 10:358–64 [PubMed: 24633353]
101. Piekna-Przybylska D, Sullivan MA, Sharma G, Bambara RA. 2014 U3 region in the HIV-1 genome adopts a G-quadruplex structure in its RNA and DNA sequence. *Biochemistry* 53:2581–93 [PubMed: 24735378]

102. Wang Y, Patel DJ. 1993 Solution structure of a parallel-stranded G-quadruplex DNA. *J. Mol. Biol* 234:1171–83 [PubMed: 8263919]
103. Kaminski JJ, Schattgen SA, Tzeng TC, Bode C, Klinman DM, Fitzgerald KA. 2013 Synthetic oligodeoxynucleotides containing suppressive TTAGGG motifs inhibit AIM2 inflammasome activation. *J. Immunol* 191:3876–83 [PubMed: 23986531]
104. Dempsey A, Bowie AG. 2015 Innate immune recognition of DNA: a recent history. *Virology* 479–80:146–52
105. Lee KG, Kim SS, Kui L, Voon DC, Mauduit M, et al. 2015 Bruton's tyrosine kinase phosphorylates DDX41 and activates its binding of dsDNA and STING to initiate type 1 interferon response. *Cell Rep* 10:1055–65 [PubMed: 25704810]
106. Jiang Y, Zhu Y, Qiu W, Liu YJ, Cheng G, et al. 2017 Structural and functional analyses of human DDX41 DEAD domain. *Protein Cell* 8:72–76 [PubMed: 27928732]
109. Cario E 2008 Barrier-protective function of intestinal epithelial Toll-like receptor 2. *Mucosal Immunol* 1(Suppl. 1):S62–66 [PubMed: 19079234]
110. Iwasaki A 2010 Antiviral immune responses in the genital tract: clues for vaccines. *Nat. Rev. Immunol* 10:699–711 [PubMed: 20829886]
111. Kijpittayarit S, Eid AJ, Brown RA, Paya CV, Razonable RR. 2007 Relationship between Toll-like receptor 2 polymorphism and cytomegalovirus disease after liver transplantation. *Clin. Infect. Dis* 44:1315–20 [PubMed: 17443468]
112. Carty M, Bowie AG. 2010 Recent insights into the role of Toll-like receptors in viral infection. *Clin. Exp. Immunol* 161:397–406 [PubMed: 20560984]
113. Hutchens MA, Luker KE, Sonstein J, Nunez G, Curtis JL, Luker GD. 2008 Protective effect of Toll-like receptor 4 in pulmonary vaccinia infection. *PLOS Pathog* 4:e1000153 [PubMed: 18802464]
114. Smith AE, Helenius A. 2004 How viruses enter animal cells. *Science* 304:237–42 [PubMed: 15073366]
115. Dimitrov DS. 2004 Virus entry: molecular mechanisms and biomedical applications. *Nat. Rev. Microbiol* 2:109–22 [PubMed: 15043007]
117. Leifer CA, Kennedy MN, Mazzoni A, Lee C, Kruhlak MJ, Segal DM. 2004 TLR9 is localized in the endoplasmic reticulum prior to stimulation. *J. Immunol* 173:1179–83 [PubMed: 15240708]
118. Hacker H, Mischak H, Miethke T, Liptay S, Schmid R, et al. 1998 CpG-DNA-specific activation of antigen-presenting cells requires stress kinase activity and is preceded by non-specific endocytosis and endosomal maturation. *EMBO J* 17:6230–40 [PubMed: 9799232]
119. Ewald SE, Lee BL, Lau L, Wickliffe KE, Shi GP, et al. 2008 The ectodomain of Toll-like receptor 9 is cleaved to generate a functional receptor. *Nature* 456:658–62 [PubMed: 18820679]
120. Park B, Brinkmann MM, Spooner E, Lee CC, Kim YM, Ploegh HL. 2008 Proteolytic cleavage in an endolysosomal compartment is required for activation of Toll-like receptor 9. *Nat. Immunol* 9:1407–14 [PubMed: 18931679]
121. Sepulveda FE, Maschalidi S, Colisson R, Heslop L, Ghirelli C, et al. 2009 Critical role for asparagine endopeptidase in endocytic Toll-like receptor signaling in dendritic cells. *Immunity* 31:737–48 [PubMed: 19879164]
122. Ewald SE, Engel A, Lee J, Wang M, Bogoy M, Barton GM. 2011 Nucleic acid recognition by Toll-like receptors is coupled to stepwise processing by cathepsins and asparagine endopeptidase. *J. Exp. Med* 208:643–51 [PubMed: 21402738]
123. Xu RH, Wong EB, Rubio D, Roscoe F, Ma X, et al. 2015 Sequential activation of two pathogen-sensing pathways required for type I interferon expression and resistance to an acute DNA virus infection. *Immunity* 43:1148–59 [PubMed: 26682986]
124. Spooner RA, Smith DC, Easton AJ, Roberts LM, Lord JM. 2006 Retrograde transport pathways utilised by viruses and protein toxins. *Virol. J* 3:26 [PubMed: 16603059]
126. West AP, Khoury-Hanold W, Staron M, Tal MC, Pineda CM, et al. 2015 Mitochondrial DNA stress primes the antiviral innate immune response. *Nature* 520:553–57 [PubMed: 25642965]
127. White MJ, McArthur K, Metcalf D, Lane RM, Cambier JC, et al. 2014 Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. *Cell* 159:1549–62 [PubMed: 25525874]

128. Rongvaux A, Jackson R, Harman CC, Li T, West AP, et al. 2014 Apoptotic caspases prevent the induction of type I interferons by mitochondrial DNA. *Cell* 159:1563–77 [PubMed: 25525875]
129. Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, et al. 2012 Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* 36:401–14 [PubMed: 22342844]
130. Sui H, Zhou M, Imamichi H, Jiao X, Sherman BT, et al. 2017 STING is an essential mediator of the Ku70-mediated production of IFN- λ 1 in response to exogenous DNA. *Sci. Signal* 10:eaah5054 [PubMed: 28720717]
131. Orzalli MH, Broekema NM, Diner BA, Hancks DC, Elde NC, et al. 2015 cGAS-mediated stabilization of IFI16 promotes innate signaling during herpes simplex virus infection. *PNAS* 112:E1773–81 [PubMed: 25831530]
132. Yang H, Wang H, Ren J, Chen Q, Chen ZJ. 2017 cGAS is essential for cellular senescence. *PNAS* 114:E4612–20 [PubMed: 28533362]
133. Biolatti M, Dell'Oste V, Pautasso S, von Einem J, Marschall M, et al. 2016 Regulatory interaction between the cellular restriction factor IFI16 and viral pp65 (pUL83) modulates viral gene expression and IFI16 protein stability. *J. Virol* 90:8238–50 [PubMed: 27384655]
134. Li T, Chen J, Cristea IM. 2013 Human cytomegalovirus tegument protein pUL83 inhibits IFI16-mediated DNA sensing for immune evasion. *Cell Host Microbe* 14:591–99 [PubMed: 24237704]
135. Cristea IM, Moorman NJ, Terhune SS, Cuevas CD, O'Keefe ES, et al. 2010 Human cytomegalovirus pUL83 stimulates activity of the viral immediate-early promoter through its interaction with the cellular IFI16 protein. *J. Virol* 84:7803–14 [PubMed: 20504932]
136. Dell'Oste V, Gatti D, Gugliesi F, De Andrea M, Bawadekar M, et al. 2014 Innate nuclear sensor IFI16 translocates into the cytoplasm during the early stage of in vitro human cytomegalovirus infection and is entrapped in the egressing virions during the late stage. *J. Virol* 88:6970–82 [PubMed: 24696486]
137. Kalamvoki M, Du T, Roizman B. 2014 Cells infected with herpes simplex virus 1 export to uninfected cells exosomes containing STING, viral mRNAs, and microRNAs. *PNAS* 111:E4991–96 [PubMed: 25368198]
138. Wu JJ, Li W, Shao Y, Avey D, Fu B, et al. 2015 Inhibition of cGAS DNA sensing by a herpesvirus virion protein. *Cell Host Microbe* 18:333–44 [PubMed: 26320998]
139. Peters NE, Ferguson BJ, Mazzon M, Fahy AS, Krysztofinska E, et al. 2013 A mechanism for the inhibition of DNA-PK-mediated DNA sensing by a virus. *PLOS Pathog* 9:e1003649 [PubMed: 24098118]
140. Stack J, Haga IR, Schroder M, Bartlett NW, Maloney G, et al. 2005 Vaccinia virus protein A46R targets multiple Toll-like-interleukin-1 receptor adaptors and contributes to virulence. *J. Exp. Med* 201:1007–18 [PubMed: 15767367]
141. Ma Z, Jacobs SR, West JA, Stopford C, Zhang Z, et al. 2015 Modulation of the cGAS-STING DNA sensing pathway by gammaherpesviruses. *PNAS* 112:E4306–15 [PubMed: 26199418]
143. Jacobs SR, Damania B. 2012 NLRs, inflammasomes, and viral infection. *J. Leukoc. Biol* 92:469–77 [PubMed: 22581934]
- 143a. Johnston JB, Barrett JW, Nazarian SH, Goodwin M, Ricciuto D, et al. 2005 A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity* 23:587–98 [PubMed: 16356857]
144. Stehlik C, Krajewska M, Welsh K, Krajewski S, Godzik A, Reed JC. 2003 The PAAD/PYRIN-only protein POP1/ASC2 is a modulator of ASC-mediated nuclear-factor- κ B and pro-caspase-1 regulation. *Biochem. J* 373:101–13 [PubMed: 12656673]
145. Liu Y, Li J, Chen J, Li Y, Wang W, et al. 2015 Hepatitis B virus polymerase disrupts K63-linked ubiquitination of STING to block innate cytosolic DNA-sensing pathways. *J. Virol* 89:2287–300 [PubMed: 25505063]
146. Inn KS, Lee SH, Rathbun JY, Wong LY, Toth Z, et al. 2011 Inhibition of RIG-I-mediated signaling by Kaposi's sarcoma-associated herpesvirus-encoded deubiquitinase ORF64. *J. Virol* 85:10899–904 [PubMed: 21835791]

147. Orzalli MH, DeLuca NA, Knipe DM. 2012 Nuclear IFI16 induction of IRF-3 signaling during herpesviral infection and degradation of IFI16 by the viral ICP0 protein. *PNAS* 109:E3008–17 [PubMed: 23027953]
148. Cuchet-Lourenco D, Anderson G, Sloan E, Orr A, Everett RD. 2013 The viral ubiquitin ligase ICP0 is neither sufficient nor necessary for degradation of the cellular DNA sensor IFI16 during herpes simplex virus 1 infection. *J. Virol* 87:13422–32 [PubMed: 24089555]
149. Diner BA, Lum KK, Javitt A, Cristea IM. 2015 Interactions of the antiviral factor interferon gamma-inducible protein 16 (IFI16) mediate immune signaling and herpes simplex virus-1 immunosuppression. *Mol. Cell. Proteom* 14:2341–56
150. Kalamvoki M, Roizman B. 2014 HSV-1 degrades, stabilizes, requires, or is stung by STING depending on ICP0, the US3 protein kinase, and cell derivation. *PNAS* 111:E611–17 [PubMed: 24449861]
151. Huang LR, Wohlleber D, Reisinger F, Jenne CN, Cheng RL, et al. 2013 Intrahepatic myeloid-cell aggregates enable local proliferation of CD8⁺ T cells and successful immunotherapy against chronic viral liver infection. *Nat. Immunol* 14:574–83 [PubMed: 23584070]
152. Guo F, Han Y, Zhao X, Wang J, Liu F, et al. 2015 STING agonists induce an innate antiviral immune response against hepatitis B virus. *Antimicrob. Agents Chemother* 59:1273–81 [PubMed: 25512416]
153. Grivennikov SI, Greten FR, Karin M. 2010 Immunity, inflammation, and cancer. *Cell* 140:883–99 [PubMed: 20303878]
154. Krieg AM. 2008 Toll-like receptor 9 (TLR9) agonists in the treatment of cancer. *Oncogene* 27:161–67 [PubMed: 18176597]
155. Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, et al. 2014 STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity* 41:830–42 [PubMed: 25517615]
156. Deng L, Liang H, Xu M, Yang X, Burnette B, et al. 2014 STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity* 41:843–52 [PubMed: 25517616]
157. Ng KW, Marshall EA, Bell JC, Lam WL. 2017 cGAS-STING and cancer: dichotomous roles in tumor immunity and development. *Trends Immunol* 39:44–54 [PubMed: 28830732]
158. Gao D, Wu J, Wu YT, Du F, Aroh C, et al. 2013 Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. *Science* 341:903–6 [PubMed: 23929945]
159. Engels EA, Biggar RJ, Hall HI, Cross H, Crutchfield A, et al. 2008 Cancer risk in people infected with human immunodeficiency virus in the United States. *Int. J. Cancer* 123:187–94 [PubMed: 18435450]
160. Herzner AM, Hagmann CA, Goldeck M, Wolter S, Kubler K, et al. 2015 Sequence-specific activation of the DNA sensor cGAS by Y-form DNA structures as found in primary HIV-1 cDNA. *Nat. Immunol* 16:1025–33 [PubMed: 26343537]
161. Horton NC, Finzel BC. 1996 The structure of an RNA/DNA hybrid: a substrate of the ribonuclease activity of HIV-1 reverse transcriptase. *J. Mol. Biol* 264:521–33 [PubMed: 8969302]
162. Du X, Poltorak A, Wei Y, Beutler B. 2000 Three novel mammalian toll-like receptors: gene structure, expression, and evolution. *Eur. Cytokine Netw* 11:362–71 [PubMed: 11022119]
163. Chuang TH, Ulevitch RJ. 2000 Cloning and characterization of a sub-family of human toll-like receptors: hTLR7, hTLR8 and hTLR9. *Eur. Cytokine Netw* 11:372–78 [PubMed: 11022120]
164. Zhang G, Chan B, Samarina N, Abere B, Weidner-Glunde M, et al. 2016 Cytoplasmic isoforms of Kaposi sarcoma herpesvirus LANA recruit and antagonize the innate immune DNA sensor cGAS. *PNAS* 113:E1034–43 [PubMed: 26811480]
165. Lau L, Gray EE, Brunette RL, Stetson DB. 2015 DNA tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway. *Science* 350:568–71 [PubMed: 26405230]
166. Sun C, Schattgen SA, Pisitkun P, Jorgensen JP, Hilterbrand AT, et al. 2015 Evasion of innate cytosolic DNA sensing by a gammaherpesvirus facilitates establishment of latent infection. *J. Immunol* 194:1819–31 [PubMed: 25595793]

DNA sensors and related signaling pathway. TLR9 recognizes CpG DNA in the endosome and recruits MyD88 to activate IRF7 and NF- κ B, leading to induction of type I interferons (IFNs) and inflammatory cytokines. AT-rich double-stranded DNA (dsDNA) is transcribed by RNA Pol III into 5'-triphosphate double-stranded RNA (5'-ppp-dsRNA), which in turn activates the RIG-I–MAVS pathway. DNA from viruses or bacteria could be detected by cGAS and potentially also by other putative DNA sensors, which are all proposed to activate the endoplasmic reticulum–resident adaptor protein STING. STING translocates from the endoplasmic reticulum to the Golgi to activate TBK1–IRF3 and NF- κ B, resulting in robust type I IFN induction and inflammatory cytokine production. Detection of DNA by IFI16 and AIM2 in the nucleus and cytoplasm, respectively, activates the inflammasome via recruitment of ASC and caspase-1, leading to proteolytic cleavage of pro-IL1 β and pro-IL18.

Table 1

Viral regulators of DNA sensors

Target type	Target	Virus	Viral protein(s)	Proposed mechanism	Reference(s)
DNA sensors	cGAS	KSHV	ORF52 (KicGAS)	cGAS interaction; DNA binding; disrupts cGAS binding to DNA	138
		KSHV	LANA	An N-terminally truncated cytoplasmic isoform of LANA interacts with cGAS	164
		MHV68	ORF52	cGAS interaction; DNA binding; disrupts cGAS binding to DNA	138
		RRV	ORF52	cGAS interaction; DNA binding; disrupts cGAS binding to DNA	138
		EBV	ORF52	cGAS interaction; DNA binding; disrupts cGAS binding to DNA	138
	IFI16	HSV-1	ICP0	Promotes the degradation(?) of IFI16 in a proteasome-dependent manner	147–149
		HCMV	pUL83	Interacts with IFI16 and blocks IFI16 oligomerization; redirects IFI16 to the major HCMV immediate early promoter	133–135
		HCMV	pUL97	Binds to IFI16 and relocalizes IFI16 to the cytoplasm	136
		VACV	C16	Binds to the Ku70-Ku80 complex and blocks DNA sensing by DNA-PK in fibroblasts	139
		HSV-1	ICP0, ICP4, US3-PK	Stabilizes STING in Hep-2 cells, which is required for optimal HSV-1 replication	150
Adaptor	STING	KSHV	vIRF1	STING interaction; disrupts STING phosphorylation; disrupts STING binding to TBK1	141
		HPV	E7	STING interaction	165
		Adenovirus	E1A	STING interaction	165
		Hepatitis B virus	Pol	STING interaction; disrupts lysine 63–linked polyubiquitination of STING	145
		VACV	A46R	Binds to MyD88 and inhibits TLR signaling	140
	MyD88	Myxoma virus	M13L	Binds to ASC1 and disrupts appropriate formation of the host inflammasome complex	143a
	ASC1	MHV68	ORF64	DUB activity loss results in stronger STING-dependent signaling	166
	?				

Abbreviations: EBV, Epstein-Barr virus; HCMV, human cytomegalovirus; HPV, human papillomavirus; HSV-1, herpes simplex virus 1; KSHV, Kaposi's sarcoma-associated herpesvirus; MHV68, murine gammaherpesvirus 68; RRV, Ross River virus; VACV, vaccinia virus.