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DNA-stimulated cell death: implications for host defence, inflammatory diseases and cancer

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Abstract

The immune system detects disturbances in homeostasis that occur during infection, sterile tissue damage and cancer. This initiates immune responses that seek to eliminate the trigger of immune activation and to reestablish homeostasis. At the same time, these mechanisms can also play a crucial role in the progression of disease. The occurrence of DNA in the cytosol constitutes a potent trigger for the innate immune system, governing the production of key inflammatory cytokines such as type I interferons and IL-1 β . More recently, it has become clear that cytosolic DNA also triggers other biological responses, including various forms of programmed cell death. In this article we review the emerging literature on the pathways governing DNA-stimulated cell death and the current knowledge on how these processes shape immune responses to exogenous and endogenous challenges.

Keywords

Cell death; DNA sensing; Innate immunology; cGAS-STING pathway

Introduction

Danger sensing and signalling is essential for eradication of infections, resolution of sterile damage and elimination of neoplasia. This allows the organism to eliminate the microbial threat, initiate tissue repair, and to reestablish homeostasis. Danger-induced responses include the expression of type I and type III interferon (IFNs), the induction of pro-inflammatory genes, the activation of inflammasome cascades, the initiation of autophagy, and also the engagement of cell death pathways¹. One major danger signal is abnormal subcellular localization of DNA (Box 1)². DNA is normally compartmentalized to the nucleus and mitochondria, yet high levels of DNA in the cytoplasm or within the endolysosomal niche stimulates danger signalling. Normally, low levels of DNA occur in these compartments, for example, during genome replication, transcription of endogenous

retroelements and phagocytosis of apoptotic cells. However, DNases located in the extracellular space (DNase I), endosomes (DNase II), and the cytoplasm (DNase III or TREX1) digest mislocalized DNA to levels below the threshold for danger signalling^{3–5}. Nevertheless, with the appearance of high amounts of exogenous DNA, for example during infections, certain thresholds seem to be passed so that DNA can initiate sensing and associated signalling cascades (Fig. 1A). This is also observed if the cellular DNases have reduced activity, or if this machinery is overwhelmed by endogenous substrates. In the past years it has emerged that the appearance of cytoplasmic DNA can also engage different types of programmed cell death (PCD) pathways and we now begin to understand the molecular basis for the interplay and cross-talk between cell death and other responses following sensing of DNA danger. In this review, we first describe key DNA sensors, and how they induce cytokine responses. This is followed by a detailed description of the current knowledge on DNA-stimulated cell death, and a discussion of how this may impact on defense and disease during infection, inflammatory diseases, and cancer.

Immune stimulation by DNA

Focus on IFN and IL-1 β —The immunostimulatory properties of DNA were first reported more than 50 years ago⁶. In particular, the ability of DNA to induce production of type I and type III IFNs and IL-1 β is a hallmark of DNA-stimulated immune responses. DNA is sensed in endolysosomes by Toll-like receptor 9 (TLR9) and in the cytoplasm by cyclic GMP-AMP (cGAMP) synthase (cGAS), and absent in melanoma 2 (AIM2), and under some circumstances also by RNA polymerase III^{7–14}.

TLR9—TLR9 was the first DNA-sensing pattern recognition receptor (PRR) to be identified⁷. Like other nucleic acid-sensing TLRs, it localizes to the endolysosomal compartment and recognizes DNA at this site¹⁵. The most potent ligand for TLR9 is unmethylated CpG DNA, which occurs abundantly in bacteria^{7,16}. TLR9 signals through the common TLR adaptor myeloid differentiation primary response protein 88 (MYD88) and activates the transcription factor IFN regulatory factor 7 (IRF7), leading primarily to IFN α production¹⁷ (Fig. 1B). TLR9 is expressed in a very cell-type restricted manner in humans, with B cells and plasmacytoid dendritic cells (pDCs) selectively expressing high levels of this PRR¹⁸. As pDCs express high levels of IRF7, TLR9 is a potent inducer of type I IFN in this cell type¹⁹.

AIM2—The second key DNA sensor to be identified was the Pyrin and HIN domain (PYHIN) family protein AIM2, which activates the inflammasome pathway^{11–14}. The inflammasome is a protein complex that initiates the cleavage of specific proteins to activate and regulate immune reactions. By far the best-studied inflammasome substrate is pro-IL-1 β , which needs to be cleaved to render bioactive IL-1 β – a central pro-inflammatory cytokine^{20,21}. AIM2 activates the inflammasome pathway in response to exogenous and endogenous DNA challenge (Fig. 1C)^{11–14}. Following DNA sensing, AIM2 recruits the adapter protein ASC (apoptosis-associated speck-like protein containing CARD), which in turn forms a filamentous structure that serves as a recruitment platform for pro-caspase-1. The recruitment of pro-caspase-1 to this structure results in its activation and thereby enables this protease to process its substrates^{21–23}. There are reports showing that another PYHIN protein IFI16, as well as NOD-, LRR- and pyrin domain-containing 3 (NLRP3), can also

induce caspase-1 activation in response to DNA ^{24,25}, the latter possibly through sensing of DNA-stimulated cell death as discussed below ²⁶. These results may suggest species-specific and cell-specific pathways for IL-1 β processing and release by DNA.

cGAS—Both of the above-mentioned DNA sensors are largely confined to the myeloid compartment, hence they cannot explain key parts of the immune response, for example, the sensing of DNA in virus-infected cells of non-myeloid origin. This notion led to an intense effort to identify cytosolic DNA sensors stimulating IFN expression, and ended with the identification of cGAS ^{8,27}. Other cytosolic DNA sensors were also proposed, but were either not independently confirmed ^{28,29}, were found to play more subordinate roles ^{9,10,30}, or were shown to be accessory proteins in the cGAS signalling pathway ^{31–33}. Upon DNA binding, the enzymatic activity of cGAS is initiated leading to production of the cyclic dinucleotide (CDN) 2'3' cGAMP ^{8,27}. This CDN is a ligand for the adaptor protein stimulator of IFN genes (STING), which in turn recruits TANK-binding kinase 1 (TBK1) ²⁷. This leads to TBK1-mediated phosphorylation at serine 366 in the carboxy-terminal tail (CTT) of STING ³⁴. IRF3 is then recruited to phospho-S366 of STING, positioning the latent transcription factor for TBK1-mediated phosphorylation and activation, eventually driving expression of *IFNA* and *IFNB* genes (Fig. 1D) ³⁵. Consequently, the CTT of STING is essential for cGAS-STING-induced IFN expression. In addition to the IRF-IFN pathway, cGAS-STING signaling leads to activation of nuclear factor- κ B (NF- κ B), and induction of inflammatory cytokines ^{26,36–38}. The mechanism of STING-mediated activation of NF- κ B remains is still poorly understood. Finally, it has recently emerged that the AIM2 pathway negatively regulates cGAS activity by, stimulating caspase 1-mediated cleavage of cGAS as well as depletion of intracellular potassium ^{39,40}. Thus, there is cross-talk between the DNA-stimulated pathways with impact on cytokine responses, but also PCD pathways as will be discussed below.

Biological importance of the DNA-IFN and DNA-IL-1 β axes—It is well documented that DNA-driven cytokine responses are of critical biological importance. Mechanistically and functionally, one can distinguish DNA-driven type I IFN responses from DNA-triggered IL-1 β maturation. For instance, control of herpes simplex virus 1 (HSV1) infection in the CNS is dependent on viral sensing and signalling by microglia through the cGAS–STING axis leading to type I IFN production ^{41–43}. For bacterial infections, the role of STING-driven IFN responses differs significantly depending on the bacterial species. For instance, although both *Legionella pneumophila* and *Listeria monocytogenes* induce type I IFN expression in a STING-dependent manner, the downstream activity of type I IFNs is protective in the case of *L. pneumophila* infection, but deleterious in the case of *L. monocytogenes* infection in mice ^{44–47}. There is also strong evidence for the importance of the IFN response triggered by endogenous DNA through the cGAS–STING pathway. For instance, individuals with TREX1 or DNase II deficiency develop autoinflammatory diseases ^{48,49}, and murine studies have demonstrated this to be dependent on STING and the type I IFN receptor ^{4,50–52}. Recently, it was reported that the release of endogenous DNA, as it occurs under acute conditions such as myocardial infarction, also triggers STING-dependent IFN responses, thereby augmenting the inflammatory pathology and decreasing survival rates ⁵³. Finally, DNA-induced IFN

responses also seem to promote control of cancer. The mechanisms involved include the activation of antigen presenting cells by tumour cell DNA, which promotes anti-tumour T cell responses ^{54,55}.

DNA-induced IL-1 β is also likely to play important roles in both defence and tissue damage. Studies from mouse disease models have shown similar phenotypes in *Aim2*^{-/-} and *Il1r*^{-/-} mice in several cases. For instance, mice deficient in AIM2 have reduced expression of IL-1 β , and exhibit elevated susceptibility to *Francisella tularensis* infection, as is also observed in *Il1r*^{-/-} mice ^{56–59}. Also, host DNA released from injured cells in the lungs during influenza A virus infections induces early AIM2-dependent IL-1 β production ^{60,61}. Accordingly, AIM2-deficiency ameliorated symptoms and lung pathology, similar to the phenotype of IL-1R-deficient mice ^{61,62}. However, another study reported that AIM2-deficiency led to elevated inflammatory response to influenza A virus infection ⁶⁰. This issue remains to be resolved. Regarding autoinflammatory diseases, there is evidence supporting the idea that the pathological IL-1 β response in psoriasis is driven by the AIM2 inflammasome ⁶³. Finally, chemotherapy causes severe gastrointestinal tract toxicity in a manner dependent on DNA damage and AIM2-induced IL-1 β production, although the total dependence on AIM2 is somewhat surprising given the presumed release of multiple DAMPs during cell lysis ⁶⁴. Collectively, the DNA–IFN and DNA–IL-1 β axes play important roles in host defence and inflammatory diseases.

Pathways for DNA-stimulated cell death

As DNA sensors have primarily been regarded as PRRs, the main focus has been put on their cytokine outputs and other outcomes that depend on *de novo* gene expression. However, in recent years it has emerged that DNA sensing also induces additional cellular events, such as autophagy ^{65–67} (Fig. 2A). In addition, it has been shown that cytosolic DNA stimulates signalling pathways that lead to different types of PCD (Box 2). These include pathways that induce apoptosis, pyroptosis, necroptosis as well as lysosomal cell death ^{12,14,68–70}. The focus of this review is to describe the current knowledge on DNA-driven cell death (Fig. 2B), highlighting the pathways involved and their physiological relevance.

The AIM2 pathway in pyroptosis and apoptosis—One of the first reports linking DNA recognition to the induction of cell death was provided by Stacey and colleagues ⁷¹. These authors observed a dramatic decline in the viability of murine macrophages upon electroporation with different types of DNA molecules. Interestingly, the observed cell death did not show hallmarks of apoptosis, indicating that another cell death modality might be at play. In retrospect, this study provided a functional characterization of the first cytosolic DNA receptor to be discovered, namely AIM2. As outlined above, AIM2 is a cytosolic PYHIN protein that harbors an N-terminal Pyrin domain and a C-terminal HIN200 domain. AIM2 detects cytosolic DNA of more than 70–80 bp in length in a sequence-independent manner ²², resulting in the formation of a Pyrin-domain platform that functions as a seed to recruit the universal inflammasome adapter molecule ASC, and the protease caspase 1, which cleaves gasdermin D to induce pyroptosis (Fig. 3A). In mouse macrophages, cytosolic DNA delivery mainly triggers AIM2–ASC-dependent cell death, with caspase-1-initiated

pyroptosis playing the predominant role. However, in the absence of caspase-1 and also when there are low amounts of DNA, ASC recruits and activates caspase-8, which then is the apical caspase in a subsequent apoptotic cell death response (Fig. 3A) ⁷². While this switches the quality of the cell death to apoptosis, it still represents an AIM2–ASC-dependent PCD. These findings extend the role of AIM2 and ASC beyond inflammasome activation, which might be relevant in cells types that do not express caspase-1, yet do express AIM2, ASC and caspase-8. The physiological importance of this alternative AIM2-driven PCD pathway has yet to be fully understood.

STING and apoptosis—One of the first reports that implemented STING signalling in the triggering of cell death came from studies in T cells infected with human T cell leukemia virus type 1 ⁷³. Here, it was observed that primary T cells underwent apoptosis that was dependent on the formation of a complex between IRF3 and BAX (Bcl2-associated X gene), which promotes apoptosis through the mitochondrial pathway ^{73,74}. Interestingly, this IRF3-mediated pathway of apoptosis (also known as RLR-induced IRF3-mediated pathway of apoptosis (RIPA)) operates independently of the transcriptional activity of IRF3, but still requires the presence of TBK1 ⁷⁴. This TBK1 requirement, yet the independence of IRF3 phosphorylation, might be attributed to the fact that TBK1-dependent phosphorylation of the adapter protein (STING or MAVS) is still required to allow IRF3 recruitment ³⁴. Further studies revealed that linear polyubiquitination of IRF3 by linear ubiquitin chain assembly complex (LUBAC) played a critical role in this particular cell death pathway ⁷⁵. In light of the fact that IRF3 is activated downstream of a number of signalling hubs, this type of cell death is not specific to cGAS–STING-dependent DNA recognition, but also seen in the context of RNA-activated RIG-I signalling. All in all, these results imply that cGAS–STING signalling triggers apoptosis independently of transcriptional activity through the mitochondrial apoptosis pathway (Fig 3B). Of note, most studies reporting on RIPA have been performed in fibroblast cell lines.

Another study that has reported on the capacity of STING to induce apoptosis proposed that the transcriptional activity of STING was required for apoptosis induction in T cells ⁷⁶. To this end, it was shown that the *de novo* expression of the BH3-only proteins via STING signalling strongly correlated with the sensitivity of cells to succumb to apoptosis. This pro-apoptotic gene expression programme correlated with STING expression levels, serving as a possible explanation as to why mouse T cells (which show high STING expression), but not mouse macrophages (which show low STING expression) die of apoptosis upon STING activation. Again, since the induction of pro-apoptotic BH3-only proteins is also seen for other PRR pathways ⁷⁷, this type of cell death induction is not unique to cGAS–STING signalling (Fig. 3B). Along these reports, additional studies have reported on the capacity of STING agonists to trigger apoptosis, yet mechanistic studies were not always performed that would allow the inference of the underlying mode of action. For example, prior to the discovery of STING, several studies documented the pro-apoptotic effects of the tumour vascular disrupting agent DMXAA (5,6-Dimethylxanthenone-4-acetic acid) in mice *in vitro* and *in vivo* ^{78,79}. With the discovery of DMXAA being a direct and specific agonist for mouse STING ^{80,81}, these studies retrospectively imply that STING-dependent effects were responsible for at least part of these observations.

Finally, cGAS-STING-induced type I IFN can stimulate genes that induce apoptosis in a paracrine manner⁸². Most notably, type I IFN stimulates expression of TNF-related apoptosis-inducing ligand (TRAIL), which act via death receptor 5 to induce apoptosis^{82,83} (Fig. 3C). This pathway has been demonstrated to be operative *in vivo* and to contribute to DNA-stimulated cell death and immunopathology⁸².

STING and lysosomal cell death—The CTT of STING and its capacity to recruit and activate TBK1 evolved along with the emergence of IRF3 and type I IFNs in the vertebrate system⁸⁴. In light of this observation, it seems plausible that certain STING functions do not require the CTT, the downstream kinase TBK1 or the functionality of the CTT to recruit IRF3 for its activation. For example, the profound lack of memory CD4+ T cells that is observed in patients with gain-of-function mutations in STING appears to be connected to such a CTT-independent STING functionality⁸⁵. To this end, transgenic expression of a constantly active STING molecule exerts a strong antiproliferative effect on CD4+ T cells and this functionality is also seen for a STING construct lacking the majority of the CTT that is incapable of activating TBK1⁸⁶. This indicates that canonical STING signal transduction is not required for this effect. Another, signaling-independent function of STING activation was observed in human myeloid cells. Here, STING activation leads to its translocation to lysosomes and the subsequent permeabilization of lysosomal membranes²⁶ (Fig. 3B). This in turn triggers a cell death pathway that is compatible with the concept of lysosomal cell death. STING-induced lysosomal cell death is observed in cells lacking TBK1 and IKK ϵ and also when expressing a mutant version of STING that cannot facilitate IRF3 phosphorylation via TBK1. As such it is clearly distinct from the canonical signalling function of STING. Indeed, in human myeloid cells STING-dependent lysosomal cell death leads to the secondary activation of the NLRP3 inflammasome, which is sensitive to cell membrane perturbation that is triggered by lysosomal cell death²⁶. These results are in so far intriguing as human myeloid cells do not utilize the dedicated DNA-sensing inflammasome sensor AIM2 for this purpose. Of note, in mouse embryonic fibroblasts and macrophages, STING is also translocated to the lysosome upon activation⁸⁷, yet in these cells lysosomes function to degrade STING, but not to induce cell death. As such. It appears that cell-type specific properties dictate the involvement of lysosomes in STING biology.

Finally, although not demonstrated to be dependent on DNA and cGAS, STING is involved in ER stress⁸⁸. This leads to downstream apoptosis through a mechanism dependent on the ER-resident kinase PERK and the downstream target transcription factor CCAAT-enhancer-binding protein homologous protein⁸⁸.

STING and necroptosis—A third type of cell death stimulated by DNA is necroptosis⁷⁰ (Fig. 3C). In this regard, it was reported that DNA transfection as well as STING agonists — in the context of pan-caspase inhibition — activate necroptosis in mouse fibroblasts, as well as in bone marrow-derived macrophages^{70,89}. This DNA-induced necroptosis was dependent on STING-induced tumour necrosis factor (TNF) and IFN α/β , and could also be triggered by other TNF- and type I IFN-inducing stimuli, such as RIG-I or TLR3 agonists^{70,89}. As such, under these conditions, there seems to be no requirement for a STING-specific signal to induce necroptosis. Nevertheless, it has been shown that tonic type I IFN

production, induced through the cGAS–STING pathway, generally lowers the threshold for necroptosis. This is governed by the expression of the key necroptosis executor mixed-lineage kinase domain like (MLKL)⁹⁰. In this regard, also TLR4-triggered necroptosis was affected by cGAS and STING-deficiency, which could be explained by a loss of tonic type I IFN production and an associated decrease in MLKL expression⁹⁰. Of note, while all of these *in vitro* experiments require the concomitant inhibition of caspases to render cells sensitive to STING-dependent necroptosis, *in vivo* experiments suggest that necroptosis can indeed be achieved by single STING agonism. To this end, mice treated with high doses of a STING ligand succumbed to lethal shock associated with pro-inflammatory cytokine production, which could be in part rescued by deficiency of RIPK3 or MLKL⁸⁹. Future studies will be required to address the role of this pathway in the context of physiological cGAS–STING activation.

Role of cGAS–STING in autophagy-dependent cell death—Finally, it is also possible that cytosolic DNA can induce autophagy-dependent cell death, which is a form of cell death induced in an autophagy-dependent manner, notably in response to strong inducers of autophagy⁹¹. The exact mechanisms that execute cell killing in autophagy-dependent cell death are not fully understood, but could give us key information on what governs the switch from healthy to deadly forms of autophagy. It is important to emphasize that there is currently no experimental evidence for DNA-activated autophagy-dependent cell death. However, since DNA strongly activates autophagy through both STING dependent and independent pathways^{66,67,92} and bacteria-derived CDNs trigger extensive ER-phagy⁸⁸, it seems possible that DNA could induce cell death through autophagy under some conditions and in some cell types.

In summary, the accumulation of DNA in the cytoplasm can promote signalling leading to many distinct types of cell death. At this stage there is limited knowledge on the factors that determine which of the death pathways are activated in a given cell type and also how the DNA-stimulated death pathways interact with other DNA-stimulated reactions, and host responses in general.

Biological functions of different types of cell death

The different types of cell death differ with respect to how they impact on host responses to danger sensing (Table 1). Most notably, apoptosis is typically understood to be non-inflammatory in nature, due to the lack of release of cellular content, and the rapid elimination by phagocytic cells. Interestingly, the avoidance of DNA recognition plays an important role in this process. To this end, a recent study has shown that tissue-resident macrophages are programmed to clear apoptotic cells in an immunologically silent manner, due to several features of these cells, including high expression of apoptotic cell recognition receptors and low expression of TLR9⁹³. Moreover, also cell-intrinsic properties function to avoid activation of the immune system by DNA in the course of apoptosis. As such, the non-inflammatory nature of apoptosis proceeds despite the release of mitochondrial DNA into the cytoplasm in the context of mitochondrial outer membrane permeabilization, which could in principle be sensed by cGAS^{94,95}. Indeed, apoptotic caspases block induction of type I IFN through the cGAS–STING pathway following release of mitochondrial DNA

^{94,95}, whereas the exact substrates in the cGAS–STING pathway that are targeted by the apoptotic caspases remain to be identified. Thus, apoptotic caspases both induce cell death and prevent the dying cells from activating a DNA-driven IFN response. Despite the generally non-inflammatory nature of apoptosis, it is important to note that apoptotic cells can undergo secondary necrosis ⁹⁶. Thus, if not rapidly eliminated by phagocytes, apoptosis can lead to inflammation through secondary necrosis. Of note, recent studies have blurred the definitions of apoptotic signalling and programmed necrosis. For certain cell types it has been shown that caspase-3 can cleave gasdermin E (also known as DFNA5), which then results in pyroptotic cell death with the associated release of cytoplasmic content ^{97,98}. Therefore, determined by multiple factors, including the expression of gasdermin E, it appears plausible that apoptotic signalling cascades can evoke mixed types of programmed cell death. Collectively, if apoptotic cells are not properly eliminated or if apoptotic cells do not prevent the cell-autonomous recognition of danger-associated molecular patterns (DAMPs), apoptosis can stimulate inflammation.

Although necroptosis and pyroptosis are induced by different pathways, they are both necrotic types of cell death with membrane rupture and release of intracellular content. One important difference between necroptosis and pyroptosis is that the latter is associated with release of inflammasome-cleaved bioactive cytokines, most notably IL-1 β ^{20,21}. Like pyroptosis and necroptosis, lysosomal cell death is also necrotic with plasma membrane rupture and release of cytoplasmic content, including DAMPs. It remains unknown whether cells dying through these different types of programmed necrosis differentially allow release of intracellular content, thereby resulting in a different repertoire of potential DAMP molecules being released.

Autophagy-dependent cell death is characterized by extensive cytoplasmic vacuolization eventually leading to phagocytic uptake and lysosomal degradation ⁹¹. As such, this type of cell death is most likely intermediate between apoptosis and different forms of necrosis with respect to stimulation of inflammation. Thus, DNA-stimulated cell death can contribute to both promotion and resolution of inflammation depending on the biological context.

Biological impact of DNA-stimulated PCD

Regarding the role of DNA-activated cell death pathways in defence and disease, there is now accumulating evidence for these pathways playing important roles in both the promotion and control of pathology (Fig. 4). For most studies, it is difficult to discern the contribution of DNA-driven cytokine responses from the PCD pathways being activated, as genetic tools to uncouple these two responses from one another are often not available. At the same time, it still remains challenging to ascertain a certain type of cell death in *in vivo* settings. In the following section, we try to provide examples in which the distinction between direct PRR signalling effects and PCD cascades was addressed.

Microbial infections—In response to infections, human T cell leukemia virus type 1 induces apoptosis in human macrophages through STING-dependent triggering of IRF3–BAX complexes ⁷³. Given the reported role for myeloid cells in spread of virus to CD4+ T cells ⁹⁹, the depletion of the infected monocytes and myeloid precursor DCs via DNA-

dependent PCD may function to prevent the spread of the virus and hence limit infection. In a study on *Mycobacterium bovis*, it was found that the bacterium induces apoptosis in murine macrophages in a manner dependent on both ER stress and STING–TBK1–IRF3¹⁰⁰. Importantly, inhibiting TBK1 blocked mitochondrial apoptosis, and elevated bacterial replication, thus suggesting DNA induced PCD to block microbial spread. The cGAS–STING pathway was also demonstrated to mediate apoptosis-like cell death in human foreskin fibroblasts infected with HSV-1, as measured by PARP cleavage¹⁰¹. However, since lysosomal cell death also leads to PARP cleavage, the published data did not resolve whether this occurs through the STING–IRF3–BAX pathway or the STING-induced lysosomal cell death pathway. It will be interesting to learn whether the cGAS–STING-dependent stimulation of apoptosis-like PCD impacts on control of herpesvirus infections. The observation that the HSV-1 ubiquitin E3 ligase ICP0 prevents cGAS–STING-dependent PARP cleavage¹⁰¹ suggests this to be the case.

Necrotic types of PCD are also induced during infections through DNA-stimulated pathways. During infections with many bacteria, pyroptosis is induced by AIM2 and/or NLRP3^{57,102–105}. This includes infection with *L. monocytogenes*, *L. pneumophila*, *Francisella tularensis*, *Streptococcus pneumoniae* and *Brucella abortus*. There is also evidence for activation of DNA-stimulated pyroptosis by viruses^{106–108} and intracellular parasites¹⁰⁹. Although it has been reported that pyroptosis can be a central player in antibacterial defence in vivo¹¹⁰, the importance of DNA-triggered pyroptosis in antimicrobial defense is still not fully established. To this end, it is currently difficult to discern the relevance of inflammasome-dependent cytokine maturation versus the induction of pyroptosis. Regarding viruses, one report has shown an antiviral role of AIM2-stimulated pyroptosis during infection with enterovirus A71 in a neuronal-like cell line¹⁰⁶. Many bacteria and viruses evade the DNA-stimulated inflammasomes, thus underscoring the importance of these pathways in antimicrobial defence. Examples of such infections include *F. novicida*, which uses a CRISPR/Cas system to maintain membrane integrity and limit AIM2 activation¹¹¹, and the HSV1 VP22 protein that binds AIM2 and inhibits downstream signalling¹¹². However, pyroptosis can also contribute to infection pathology. For instance, the observation that *Casp1*^{−/−}*Casp11*^{−/−} mice are protected against *Escherichia coli* sepsis whereas mice double-deficient in IL-1 β and IL-18 are not, suggests that pyroptotic cell death amplifies the inflammatory response in a pathological manner¹¹³. A DNA-dependent example is provided by HIV, which infects CD4⁺ T cells and eventually causes T cell depletion and immunodeficiency. Greene and coworkers demonstrated that the AIM2-family member IFI16 senses DNA in cells abortively infected with HIV, thus inducing pyroptosis, and potentially contributing to T cell depletion, chronic immune activation and disease pathogenesis¹⁰⁷.

Regarding the role of DNA-driven necroptosis during infections there is only limited information available. However, as outlined above, it has been reported that hyper-activation of STING in mice leads to a shock-like syndrome, which is dependent on TNF and IFN α/β , both of which are also required for STING-dependent necroptosis⁸⁹. These data suggest that STING signalling might also engage necroptosis under physiological conditions, such as infections with herpesviruses, which potently activate cGAS–STING signalling^{43,114}. Importantly, murine gammaherpesvirus MHV68, which is closely related to Epstein-Barr

virus and Kaposi's sarcoma-associated herpesvirus, induces necroptosis in a murine fibroblast cell line in a manner dependent STING and TNF70. Since several DNA viruses that are known to trigger the cGAS–STING pathway also encode proteins that can inhibit caspase 8, for example vaccinia virus ¹¹⁵, it is likely that DNA-driven necroptosis is of importance for control virus infections. Indeed, *Ripk3*^{-/-} mice are more susceptible to vaccinia virus infection than wild-type mice ¹¹⁶.

Sterile inflammatory diseases—Given the potentially pro-inflammatory role of necrotic types of cell death, it would not be surprising if programmed DNA-stimulated cell pathways also contribute to the pathogenesis of sterile inflammatory diseases. This is indicated by the finding that the STING–IRF3–BAX apoptosis pathway is activated in alcoholic liver disease, and that *Irf3*^{-/-} mice but not *IFNAR*^{-/-} mice are protected from disease ¹¹⁷. In many inflammatory diseases where DNA-stimulated pathways have been reported to contribute to the pathogenesis, there is extensive cell death. This includes, systemic lupus erythematosus, Sjögren's syndrome, and STING-associated vasculopathy with onset in infancy (SAVI) ^{85,118,119}. However, at present there is limited data directly demonstrating that the cell death occurring in these pathologies is induced by DNA. Currently, the data available are indirect. For instance, STING agonists induce apoptosis in endothelial cells and disrupt vasculature ⁷⁸, which is seen in the above-mentioned diseases. These diseases are also commonly referred to as type I interferonopathies, due to the high level of type I IFNs associated with them. Interestingly, DNA-induced IFN production is subject to negative regulation by AIM2-dependent pyroptosis ^{11,120}. Therefore, lack of full AIM2 activity leads to augmented DNA-stimulated type I IFN responses due to prolonged activation of the cGAS–STING pathway. Two questions emerging from the discussion above are whether type I IFN is in fact the only main driver of the interferonopathies, and whether PCD may play a role. In favor of IFN-independent mechanisms also contributing to the pathogenesis, for at least some interferonopathies, is the finding that mice harbouring the SAVI-mimicking mutation N153S are not protected from disease in the absence of the central IFN-inducing transcription factor IRF3 ¹²¹. This is, however, in contrast to *Trex1*^{-/-} *Irf3*^{-/-} mice, which are protected from cGAS–STING dependent disease ^{5,51,122}. Since, accelerated cell death is observed in SAVI ⁸⁵, it would be interesting to test whether STING N153S mice with selective defects in STING-dependent death pathways are protected against disease.

Despite the lack of solid evidence for DNA-driven cell death in human inflammatory diseases, the concept of DNA-stimulated cell death as a key driver in sterile inflammation has been demonstrated. Flavell and associates reported that radiation-induced tissue damage was dependent on DNA-induced cell death ¹²³. Importantly *Aim2*^{-/-} mice were protected against both subtotal body irradiation-induced gastrointestinal syndrome and total body irradiation-induced hematopoietic failure, and the effect of AIM2 was largely mediated by pyroptotic cell death. As another example of DNA-driven cell death in acute sterile inflammation, it was reported that Aortic aneurysm leads to STING-dependent activation of TBK1, RIPK3 and phospho-MLKL in smooth muscle cells. STING deficiency or TBK1 inhibition blocked these responses and improved the disease outcome in mice. These data suggest a role for STING dependent necroptosis in aortic aneurysm ¹²⁴. Similar observation

have been made in a mouse model for inflammatory bowel disease, where STING was found to drive type I IFN and TNF α -mediated necroptosis of intestinal epithelial cells¹²⁵.

Cancer—Cancer is characterized by uncontrolled cell proliferation but also excessive cell turnover. This leads to accumulation of host DNA in the cytoplasm, which can stimulate beneficial and pathological signalling³⁷. In the process of proliferation of cancer cells, micronuclei are frequently formed in the cytoplasm, and these can be detected by cGAS^{126,127}. This could suggest a role for the cGAS–STING pathway, and maybe cytosolic DNA sensing in general in tumour immunity. In line with this, many cancer cells have lost STING expression, and clear effects of STING gene deletion or STING agonists on cancer development have been reported^{54,55,128–130}. On the other hand, cytosolic DNA may also drive innate immune responses that promote cancer progression^{37,131}.

The ability of the STING pathway to induce apoptosis or apoptosis-like cell death can directly target STING-expressing tumour cells for death following accumulation of DNA in the cytoplasm. In mouse models for T cell leukemia and multiple myeloma, treatment with STING agonists induces apoptosis in cancer cells and this promotes tumour control and improves disease outcome^{76,132}. These data reveal a therapeutic potential for CDNs as direct tumoricidal agents against STING-expressing tumour cells. It will be interesting to learn whether cell-autonomous apoptosis induced by endogenous DNA derived from tumour cells is involved in tumour immunosurveillance. On the other hand, since the T cell response is a major player in tumour immunity, apoptosis of lymphocytes may also hamper the anti-tumour immune response. To this end, it has been reported that STING agonists induce tolerogenic effects on T cell responses, so there is a need to understand this phenomenon in more detail¹³³.

Many of the current cancer therapies target cancer cells through either irradiation or inhibition of DNA replication. As discussed above irradiation induces AIM2 activation and pyroptotic cell death¹²³. In addition, chemotherapeutic agents such as cisplatin and etoposide inhibit DNA replication³⁷. Thus, the mechanisms through which conventional anti-cancer therapies kill cancer cells may involve DNA-activated PCD pathways. Therefore, altered expression of proteins in the DNA-sensing death pathway by cancer cells may not only affect tumour growth, but could also impact on the efficiency of therapy.

Mechanistic understanding of DNA-stimulated cell death pathways and their role in human pathologies may allow development of new treatments. There is currently a large interest in development and therapeutic use of agonists and antagonists for the cGAS–STING pathway, and significant progress has already been made^{134–137}. The field is thus in a favorable position to rapidly take advantage of such new discoveries, to potentially target DNA-triggered PCD in a number of diseases.

Concluding remarks and future outstanding questions

In recent years it has become clear that cells can succumb to cell death through a broad range of programmed mechanisms. At the same time, it has become apparent that these pathways constitute a central part of the immune response to infections and also the

restoration of homeostasis under sterile conditions. Accumulation of cytoplasmic DNA, which occurs during infections, sterile tissue damage, autoimmune diseases, and cancer, is a very potent danger signal that has mainly been reported to induce the production of cytokines such as type I IFNs and IL-1 β ². However, as outlined in this review, cytoplasmic DNA also induces a number of PCD pathways, which we are now starting to understand at the molecular level. Furthermore, there is accumulating evidence for these pathways being of relevance for the beneficial and pathological biological activities of cytoplasmic DNA. With the recent advances in this field, a number of outstanding questions have emerged. For instance, what determines whether DNA stimulates type I IFNs, IL-1 β , apoptosis, autophagy, necroptosis, lysosomal cell death and/or pyroptosis? Possible influencing factors include; the strength of signal induced by DNA recognition, the duration of signalling, the cell type sensing DNA, the length and format of the DNA, whether there is microbial modulation of specific DNA-sensing pathways, the metabolic state of the host, and the local cytokine environment. Also, we still do not understand many of the molecular details of the pathways for DNA-stimulated PCD. In addition, much more work and better tools are required to fully characterize the roles of the different DNA-stimulated PCDs in diseases.

On a final note, the discovery that cytosolic DNA can induce multiple types of PCD has revealed the ironic phenomenon that the immune system uses ‘the molecule of life’ to induce death in many different ways.

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DNA as a molecule stimulating innate immune responses

DNA is the molecule carrying the genetic code of living organisms. However, DNA also has other properties, including being the main constituent of neutrophil extracellular traps¹³⁸, and being a danger signal for the innate immune system⁶. DNA gains immunostimulatory activity when localized in abnormal cellular compartments, notably endosomes, the cytoplasm, or micronuclei^{7,126,127,139,140}. In the original definition by Charlie Janeway, pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs), which are (i) conserved among entire classes of pathogens, (ii) essential for the survival of the pathogen, and (iii) different from “self”¹⁴¹. In addition, the term danger-associated molecular patterns (DAMPs) has been coined, which is usually understood to represent endogenous molecules that gain PRR-agonistic activity upon cellular stress or damage. Based on these criteria, microbial DNA is not a bona fide PAMP, but falls into the broader category of microbe-associated molecular patterns. By contrast, host-derived DNA fulfils the criteria for the term DAMP.

Regarding the sensing of double-stranded (ds) DNA by PRRs at the molecular level, the cytosolic DNA sensors AIM2 and cGAS detect dsDNA through binding to the sugar-phosphate backbone in a sequence-independent manner^{22,142–144}. For AIM2, this is achieved through a positively charged surface in the carboxy terminal HIN domain, whereas cGAS uses a positively charged surface as well as the zinc thumb to interact with the DNA backbone. For both AIM2 and cGAS, immune activation is DNA length dependent^{9,14,31,145}, and in the case of cGAS it was recently reported that under conditions with low DNA concentrations, long dsDNA species (>1000 bps) are much more stimulatory and with much lower concentration thresholds¹⁴⁶. This argues for long DNA species being the physiological trigger of DNA-induced immune responses.

Modalities of cell death induced by DNA

Various forms of cell death can be induced in a programmed manner through specific signalling pathways initiated by a range of triggers, including DNA.

Apoptosis

Apoptosis was first observed more than 175 years ago by the zoologist Klaus Vogt studying development of the tadpole, and later named apoptosis by Kerr, Wyllie and Currie in 1972¹⁴⁷. Apoptosis displays a number of biochemical and morphological characteristics, including cell shrinkage, membrane blebbing, chromatin fragmentation, and mRNA decay. Apoptosis can be induced through pathways relaying signals from plasma membrane receptors (extrinsic pathway) or from the mitochondria (intrinsic pathway)¹⁴⁸. Both pathways converge in the activation of the effector caspases, caspase 3 and caspase 7, with the extrinsic pathway mainly utilizing the initiator caspase 8 and the intrinsic pathway caspase 9¹⁴⁹. Apoptotic cell death generates cell fragments called apoptotic bodies, which are phagocytosed and removed by macrophages. This functionality also, functions to hinder the spread of microorganisms and the release of immunostimulatory molecules¹⁴⁷. If not removed, apoptotic cells can undergo secondary necrosis^{96,150}.

Pyroptosis

One prominent form of programmed necrotic cell death is pyroptosis. The term was coined by Cookson and Brennan following the description of rapid caspase 1-dependent cell death in *Salmonella*-infected macrophages^{151–153}. Pyroptosis is initiated by so-called inflammatory caspases that encompass caspase-1, which is activated in the context of inflammasome complexes, or by caspases that are activated in response to cytosolic LPS, namely caspase 4 and caspase 5 in humans (or caspase 11 in mice). These proteases in turn cleave gasdermin D, thus generating an N-terminal fragment that localizes to the inner leaflet of the plasma membrane where it forms large pores. These pores result in osmotic swelling of cells and subsequent rupture of the plasma membrane^{154,155}. Thereby cytosolic content is released, which may act as DAMP molecules that trigger inflammation. Results from negative stain electron-, cryo electron, and atomic force-microscopy have shown that the Gasdermin pore has an inner diameter of 14–20 nm^{156–158}. Such a pore size allows passage of larger molecules, including mature IL-1 β and caspase-1, with diameters of 4.5 and 7.5 nm, respectively.

While pyroptosis was initially considered to be restricted to the activation of the pro-inflammatory caspases, more recent evidence suggests that pyroptosis can also be executed in the context of apoptotic caspases. As such, the Gasdermin family member gasdermin E (also known as DFNA5) can be cleaved by the apoptotic effector caspase 3 to effectuate pyroptotic cell death in a manner analogous to gasdermin D^{97,98}. Consequently, pyroptosis has to be defined as a cell death that is carried out by gasdermin pores, rather than by a specific pattern recognition receptor (PRR) cascade or caspase¹⁵⁹.

Necroptosis

Necroptosis is like pyroptosis a necrotic form of cell death that encompasses the disintegration of the plasma membrane and the release of cytosolic content. It was initially identified through studies where cells were treated with death receptor agonists in the presence of pan-caspase inhibitors¹⁶⁰. Necroptosis is induced by a number of pathways (for example, the RIPK1, TRIF or ZBP1 pathways) that converge upon the activation of receptor-interacting serine/threonine-protein kinase 3 (RIPK3). RIPK3, in turn, phosphorylates the pseudokinase protein MLKL (mixed lineage kinase domain-like protein) leading to a change in its conformation, translocation to the plasma membrane and subsequent membrane disruption^{161–163}. Activation of caspase 8 negatively regulates necroptosis¹⁶⁴, and thus necroptosis has been suggested to be a type of ‘back-up’ death pathway in cells where the apoptosis pathway is blocked, as can occur for instance during virus infections. It should be noted that most knowledge on necroptosis has been performed *in vitro*, and it remains to be thoroughly investigated to which extent necroptosis occurs *in vivo*.

Lysosomal cell death

Christian de Duve described lysosomal cell death more than 40 years ago. Lysosomal membrane permeabilization and the release of lysosomal hydrolases (e.g. cathepsins) occurs secondary to many forms of PCD. However, lysosomal membrane permeabilization can also function as the primary initiator of cell death. Here, the release of activated lysosomal hydrolases into the cytoplasm leads to the engagement of various redundant, yet ill-defined pathways that result in the effectuation of cell death¹⁶⁵. Indeed, attempts to define lysosomal cell death by genetic means have been unsuccessful, attributable to the redundancy of cell death inducing triggers originating from the leakage of lysosomal hydrolases into the cytoplasm. In this regard it is conceivable that lysosomal enzymes directly serve as the death-executing proteases. Lysosomal cell death typically displays a necrotic phenotype with plasma membrane permeabilization, with caspase inhibition showing only little effect. Nevertheless, despite the caspase-independent nature of lysosomal cell death, several characteristics of apoptosis, such as the cleavage of caspase 3 can be observed²⁶.

Figure 1. Hallmark cytokine responses induced by DNA-sensing PRRs.

(A) DNA originating from microbes, mitochondria, phagocytosed apoptotic cells, or leaked from the nucleus can accumulate in the cytoplasm where it is sensed by pattern recognition receptors. This initiates danger signaling. (B-D) Type I IFN and IL-1 β production are the most studied immunological responses downstream of cytosolic DNA. (B) TLR9 senses CpG-rich DNA in endosomes and signals via MyD88 to activate IRF7, which is abundantly expressed in plasmacytoid dendritic cells. This leads to strong activation of genes encoding IFN α . (C) dsDNA in the cytoplasm is also sensed by AIM2, which promotes assembly of inflammasomes with caspase 1 activation, and downstream cleavage of pro-IL-1 β to the bioactive cytokine. (D) dsDNA is sensed by cGAS, leading to enzymatic activation and synthesis of the STING agonist 2'3'cGAMP, thus leading to activation of the kinase TBK1, phosphorylation of the transcription factor IRF3, and transcriptional activation of the promoters for the type I (α/β) and type III (λ) IFN genes. Abbreviations not defined in the text: HTLV-I, Human T-lymphotropic virus I; IKK, I κ B kinase; IRAK, Interleukin-1 receptor-associated kinase; Trex, Three prime repair exonuclease.

Figure 2. Cytoplasmic DNA activates a broad range of stress responses.

(A) The cellular responses induced by cytoplasmic DNA includes, in addition to production of type I IFN and IL1 β , a range of other activities. This includes NF- κ B-induced gene expression, autophagy, and a panel of PCD pathways, such as apoptosis, necroptosis, and pyroptosis. (B) Overview of the current knowledge on PCD induced by cytoplasmic DNA, including the interactions between the pathways. Abbreviations not defined in the text: ULK, Unc-51 like autophagy activating kinase.

Figure 3. Pathways in DNA-stimulated cell death.

(A) Cell death pathways stimulated by AIM2 in response to DNA sensing. (B) Cell-autonomous death pathways stimulated through STING-dependent mechanisms. (C) Stimulation of apoptosis and necroptosis by STING-dependent paracrine signaling. Abbreviations not defined in the text: IFNAR, IFN α receptor; ISGF, IFN-stimulated gene factor; NF, nuclear factor; PUMA, p53 upregulated modulator of apoptosis; RIPA, RLR-induced IRF-3-mediated pathway of apoptosis; TNFR, TNF receptor.

Figure 4. Role of DNA-stimulated programmed cell death in pathology and host protection. Current understanding of the role of DNA-stimulated apoptosis, necroptosis, and pyroptosis in defense against infections and cancers, promotion of immunopathology, and regulation of immune responses. For more details see the text.

Table 1
Features of types of cell death induced by cytoplasmic DNA

Type of cell death	Kinetics	Plasma Membrane	Characteristics	Executor	Inflammatory
Apoptosis	Slow	Preserved integrity	DNA fragmentation Apoptotic bodies	Cleavage of caspase 3 and caspase 7 substrates	No
Pyroptosis	Fast	Rupture	Gasdermin cleavage	Gasdermin pores	Yes
Necroptosis	Slow	Rupture	MLKL phosphorylation	MLKL pores	Yes
Lysosomal cell death	Slow	Rupture [/]	Death dependent on components released from lysosomes	Unknown	Yes
Autophagic cell death	Slow	Focal rupture	Cell death dependent on autophagy	Unknown	Yes/no

[/] The degree of plasma membrane rupture depends on the extent of lysosome lysis. Upon limited lysosomal rupture, the cells may be primed to caspase-independent apoptosis-like cell death