Published in final edited form as:

Arthritis Rheumatol. 2018 June ; 70(6): 805–816. doi:10.1002/art.40452.

Cell Death, Nucleic acids and Immunity: Inflammation beyond the Grave

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Abstract

Cells of the innate immune system are rigged with sensors that detect nucleic acids derived from microbes, especially viruses. It has become clear that these same sensors that respond to nucleic acids derived from damaged cells or defective intracellular processing are implicated in triggering diseases such as lupus and arthritis. The ways in which cells die and the concomitant presence of proteins and peptides that allow nucleic acids to re-enter cells profoundly influence innate immune responses. This review briefly discusses different types of programmed necrosis such as pyroptosis, necroptosis and NETosis and explains how nucleic acids can engage intracellular receptors and stimulate inflammation. Host protective mechanisms that include compartmentalization of receptors and nucleases as well as the consequences of nuclease deficiencies are explored. Finally, we discuss proximal and distal targets in the nucleic acid stimulation of inflammation that are amenable to therapy to attenuate innate immune activation and disease pathogenesis.

Introduction

Rheumato-immunologists began paying attention to cell death after the discovery in 1992 that a mutation in the *fas* gene caused acceleration of autoimmunity in the MRLlpr lupus mouse strain (1). *Fas* encodes a cell surface death receptor, CD95, that promotes apoptosis of activated immune cells. Loss of *fas* function leads to accumulation of lymphocytes, including those with autoreactivity. Further interest in cell death was stimulated by the observation that many autoantigens redistribute to the cell surface following induction of apoptosis (2). Over the last 25 years, the importance of cell death pathways and the way in which immune cells respond to dead and dying cells has been intensively studied and shown to be important across diseases.

Early studies of cell death made a fundamental distinction between apoptosis and necrosis; apoptosis being controlled by a biochemical program and immunologically silent whereas necrosis is immediate, uncontrolled and inflammatory. While it remains true that rapid uncontrolled cell death as, for example induced by infarction, trauma or burns, leads to cell membrane damage and spillage of cell contents (necrosis), it has become apparent that

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Disclosures: The author is co-founder of Resolve Therapeutics and AMDAX Therapeutics. He has received consulting or seminar fees from Celgene, Astra Zeneca, Merck Research Laboratories, MedImmune and Bellbrook Laboratories.

necrosis can also occur by programmed biochemical pathways discussed in detail below. Involvement of executioner caspases (caspases are cysteine proteases that help degrade intracellular proteins) can also serve to distinguish apoptosis from necrosis (3). If they are involved, then death is more likely to be apoptotic, whereas failure to engage executioner caspases leads to necrosis and inflammatory death. It has also become apparent that, following necrotic forms of cell death, nucleic acids are prominent, if not dominant, activators of innate immunity. Even during apoptotic cell death, it is the presence of nucleic acids on apoptotic cells or coating the blebs released from dying cells that have the potential to engage B cell receptors and partially activate B lymphocytes.

What are PAMPs and DAMPs?

The discoveries of antigen receptors on cells of the adaptive immune system, T and B lymphocytes, as well as the fundamental principles that determine self / non-self recognition through ‘education’ in the thymus and bone marrow respectively, were elucidated in the 1980s. What are the receptors and fundamental principles of self / non-self recognition for cells of the innate immune system (here focusing particularly on macrophages and dendritic cells)? In 1989, Charles Janeway hypothesized that cells of the innate immune system also had germline encoded receptors that could recognize foreign antigens (4). However, unlike the cell surface antigen receptors on B and T cells that generally show high specificity for peptide sequences, the receptors in the innate immune system, located both on the cell surface and within cells, appeared to respond to molecular patterns in microbes that are not restricted to proteins. These receptors were called Pattern Recognition Receptors (PRR). The primary consequences of PRR engagement in innate immune cells were expression of co-stimulatory proteins, and release of chemokines and cytokines (Fig. 1). The microbial ligands, called Pathogen Associated Molecular Patterns (PAMPs) that triggered PRRs include the bacterial cell wall component lipid polysaccharide (LPS), the bacterial protein flagellin and viral nucleic acids. Challenged by evidence that inflammation can arise during tissue damage with the release of dead and dying cells in the absence of infection (sterile inflammation and ‘the danger model’ (5), concepts were expanded to include host derived ligands (Damage Associated Molecular Patterns or DAMPs) that include nucleic acids, monosodium urate (MSU) crystals and the protein, HMGB1.

Since the initial inflammatory response by the innate immune system can be identical when activated by PAMPs or DAMPs, the fundamental questions raised are whether and how the innate immune system distinguishes self from foreign? The answer, similar to the lessons learned from adaptive immunity, is that the innate immune system employs a variety of overlapping strategies that vary in time with the outcome depending on both the nature of the stimulus and the quantity and quality of the host response. With regard to self antigens released as DAMPs, how cells die plays a critical role in alerting the innate immune system to “friend or foe”.

How cells die determines non-inflammatory versus inflammatory consequences

Beyond the apoptosis / necrosis dichotomy, at least 13 different biochemical forms of cell death have been recognized (6). Different names have been given to these forms of death according to the inductive stimulus (starvation, end stage maturation, gene damage), cell type (cornification in skin cells, pyroptosis in macrophages and Neutrophil Extracellular Trap (NET) formation in neutrophils) and involvement of caspases. Here, we consider different types of cell death: apoptosis, necrosis, necroptosis, pyroptosis and NET formation since each of these types of cell death impacts inflammation and autoimmunity either negatively or positively. We then consider host cell mechanisms that function to reduce inflammation from cell-derived material. It should be emphasized that an immune response, even when involving inflammatory cytokines, is not necessarily deleterious as many of these responses are part of a wound repair process or trigger healing pathways (reviewed in (7)).

Although the outcomes in each form of cell death discussed below differ, what most have in common is the generation of a macromolecular structure or platform comprising the key effector molecules required for execution of the program (e.g. apoptosome in apoptosis, necrosome in necroptosis and inflammasome in pyroptosis). Space precludes a detailed description of the components and biochemical function of these structures but they can be found in references included in each form of cell death.

Apoptosis and post-apoptotic necrosis

Apoptosis can be triggered by extracellular signals via activation of a “death receptor,” or by intracellular events such as DNA damage, endoplasmic reticulum (ER) stress, or lack of nutrient support. Elimination of cells by apoptosis plays obligate roles in normal development, tissue homeostasis and immune regulation. Apoptosis initiated by death receptor signaling is termed extrinsic apoptosis, while that induced by intracellular stresses is termed intrinsic apoptosis. Both apoptotic pathways converge on activation of caspases, caspase 8 for the extrinsic pathway and caspase 9 for the intrinsic pathway. The ensuing proteolytic cascade leads apoptotic cells to undergo distinct morphological changes, including dismantling of the cytoskeleton, and alterations in the lipid composition of the cell membrane. This last event, especially the appearance of phosphatidylserine (PS) on the cell surface serves as a signal to phagocytes, which readily engulf apoptotic cells (a process also called “efferocytosis”) prior to the loss of membrane integrity by the dying cells. Apoptotic cells are thereby “packaged” for phagocytosis, and their contents are not released into the intracellular space. For this reason, apoptosis is generally considered a non-inflammatory and immunologically silent form of cell death. Efferocytosis may induce the stimulation of TGF- β and IL-10 which are both immunosuppressive cytokines further diminishing the chance for autoimmunity (Fig. 1).

Disease relevance—Delay in the engulfment of apoptotic cells (AC) results in two events that impact immune responses. Blebbing with the production of microparticles may occur. Microparticles (MP) are a special class of Extracellular Vesicles (EVs, that also include exosomes and microvesicles). The MP contain nucleic acids as well as surface exposed

DNA and are implicated in cell-cell communication and either functional suppression or stimulation of immune cells depending on the MP cellular origin and patient context (8, 9). A second, and very significant consequence of delayed clearance for immune homeostasis is that the cell membranes of AC break down and release their intracellular contents in the process of post-apoptotic necrosis (Fig. 2). The released nucleic acids appear to be the main source of DAMPs. However, extra- and intracellular nucleases as well as limited re-entry into cells help to prevent persistent inflammation as discussed below.

There is considerable evidence in mouse models that failure to clear AC leads to inflammation and / or autoimmunity (10). Genetic deletion of receptors or ligands that facilitate apoptotic cell clearance such as early complement components, MFGE-8, c-mer, axl and tyro, Tim-4, predisposes to lupus although disease is usually mild unless other susceptibility factors are present. There is evidence that some SLE patients have reduced clearance of AC as evidenced by an increased number of AC in their germinal centers (11), but which molecular pathways are defective is unclear. Both in vitro and in vivo experiments strongly support the idea that the early complement components are required for the clearance of apoptotic cells (12, 13). The high frequency of SLE in patients with C1q, C4 and C2 deficiency (reviewed in (14) may, in part, be explained by impaired clearance of dying cells. Inappropriately low levels of CRP in SLE may further reduce apoptotic cells clearance (15).

Pyroptosis

Pyroptosis is a form of cell death most commonly induced by bacterial PAMPs (16). These PAMPs are recognized by the PRR cytosolic sensors, leading to the assembly of multiprotein complexes called **inflammasomes** (17). Inflammasome platforms can be assembled not only by PAMPs (e.g. gram negative bacteria activate non- classical NLRP3 inflammasomes and bacterial Rho inactivating toxins activate the pyrin inflammasomes) but also by DAMPs released by stressed and dead cells (e.g. MSU crystals as well as DNA can activate the classic NLRP3 inflammasome). Inflammasome assembly in turn leads to activation of specific members of the caspase family, termed the **inflammatory caspases** (human caspases 1, 4 and 5 where human caspase-4 and 5 are functionally homologous to murine caspase 11 (reviewed in (18)).

The activation of the inflammatory caspases leads to two events that render pyroptosis distinct from apoptosis. First, the catalytic activity of caspase-1 is required for the maturation and bioactivity of two key cytokines, Interleukin-1 β (IL-1b) and IL-18 (19). These cytokines are synthesized as inactive precursors, and their cleavage by caspase-1 renders them active. Once activated and released from the cell, these cytokines are able to initiate complex and pleiotropic inflammatory events. How do these cytokines that lack the leader sequences that allow secretion of most cytokines via the Golgi apparatus exit the cell? Recently it was observed that the inflammatory caspases cleave and thereby activate the pore-forming molecule, gasdermin-D (GSDMD), an executioner of pyroptosis (20, 21). GSDMD activation leads to the disruption of the cell membrane, cellular swelling and rupture with the release of intracellular contents (Fig. 2). The lytic nature of pyroptosis stands in obvious contrast to the tidy packaging and phagocytosis associated with apoptosis.

Disease relevance—There is no definite association between gram-negative bacteria, activation of the inflammasome and pyroptosis in rheumatic disorders. Nevertheless, uric acid released by stressed or dying cells (22), MSU crystals and also intracytoplasmic DNA stimulate inflammasome platforms to generate IL-1b. Whereas hyperuricemia is largely a consequence of increased production or reduced excretion of uric acid to what extent uric acid derived from dying cells contributes to gout is not known but blockade of IL-1b with IL-1 Receptor Antagonist (IL1RA) is an effective, albeit, expensive therapy for this disease. The pyrin inflammasome is stimulated by bacterial toxins that inactivate the GTP binding protein, RhoA (23). Humans with missense mutations in the *MEFV* gene that encodes pyrin, autoactivate the pyrin inflammasome and develop familial Mediterranean fever (FMF) in significant part due to the release of IL-1b. Once again, proof of principle is provided by blockade of IL-1b with IL-1RA or antibodies to IL-1b that have been shown to be effective forms of treatment for colchicine resistant FMF (24).

Necroptosis

Necroptosis is a caspase independent form of programmed cell death morphologically similar to necrosis. Necroptotic cells swell and rupture, releasing their intracellular contents in a manner analogous to necrotic and pyroptotic cells. However, the mechanisms by which necroptosis occurs are distinct: Necroptosis is mediated by the activation of receptor-interacting protein kinase-1 (RIPK1) and RIPK3. The RIP kinases are activated by ligation of many of the same “death receptors” such as TNF-receptor or Fas (CD95) that induce the extrinsic pathway of apoptosis. Strikingly however, the molecules that induce extrinsic apoptosis suppress necroptosis. The outcome of death receptor signaling is therefore pleiotropic, and is determined by the relative abundance and activity of the signaling components that bind to the cytoplasmic tails of these receptors (25). Specifically, the relative lack of bioactive caspase 8 or increased cellular FLICE-like inhibitory protein (cFLIP_L) promote necroptosis rather than apoptosis.

Once activated, the RIP kinases phosphorylate an effector molecule, mixed lineage kinase-domain like (MLKL) which disrupts the cell membrane (26) (Fig. 2). This mechanism of programmed membrane disruption is analogous to the execution of pyroptosis by GSDMD. As a lytic form of cell death, it has been hypothesized that necroptosis may promote inflammation through the release of DAMPs, although the precise mechanisms by which this occurs remain to be determined.

Disease relevance—Necroptotic death is seen in virus infections (e.g. cytomegalovirus) that produce caspase inhibitors and has also been implicated in neurodegeneration and inflammatory bowel disease (27). Expression levels of RIPK and RIPK3 are reported to be reduced in SLE (28). In contrast, in collagen induced arthritis (CIA), these kinases were increased and could be shown to play a role in inflammation, especially in the absence of interferon-gamma (IFN-g) (29).

Neutrophils and NET formation

Neutrophils are short-lived cells that may die by apoptosis, necrosis or NET formation. Since they are the most abundant white blood cell in the circulation and are first responders

to infection and injury, their mode of death is particularly important. NET formation is a programmed form of neutrophil necrosis (30), although a similar process has been described in eosinophils and basophils. This form of rapid cell death is characterized by the extrusion of chromatin in a NET like structure accompanied by the release of neutrophil-derived enzymes and mitochondria or their fragments. NET formation is stimulated by exposure of neutrophils to certain bacteria, immune complexes (ICs) containing RNA which, in turn stimulates the intracellular Toll Like Receptor 8 (TLR8), the dominant TLR in neutrophils. Depending on the stimulus, the NET formation program requires activation of the enzyme, peptidylarginine deiminase 4 (PAD4), leading to citrullination of histone H3 and unpacking of chromatin as well as activation of NADPH oxidase or mitochondrial reactive oxygen species (ROS) generating systems.

Disease relevance—Increased generation of NETs and/or impaired NET clearance has been implicated in the pathogenesis of autoimmune disorders, including SLE, vasculitis and rheumatoid arthritis (RA) (31). In SLE, low density granulocytes undergo spontaneous NET formation *ex vivo* strongly suggesting that this process is initiated *in vivo*. Lupus patients have increased circulating levels of NETs and evidence for NET formation can also be found in tissue (32). There is some evidence that patients with SLE or vasculitis have an impaired ability to degrade NETs (33) which leads to increased inflammation and type I IFN production. These observations have been supported by mouse studies in which pharmacologic inhibition of NET formation in lupus-prone mice, either by inhibiting PAD4 activity or by inhibiting mitochondrial ROS, ameliorates disease, (34, 35). However, an argument against the requirement for NETs has been made by genetic studies in MRL.lpr mice where deficiency of either PAD4 or Nox2 did not protect against disease (36, 37). ROS generation is necessary for NET formation in most contexts and we and others recently demonstrated that ROS generation by mitochondria, rather than Nox2, is key to oxidation of DNA and in particular, mitochondrial DNA (mtDNA) (34, 38). Furthermore, oxidized mtDNA proved to be highly inflammatory through activation of the Stimulator of Interferon Genes (STING) pathway, inducing IFN- β and other inflammatory cytokines (34). In gout, neutrophils that ingest MSU crystals stimulate inflammatory cytokines and can induce NET formation. Interestingly, gouty tophi are composed, in part, of aggregated NETs which degrade cytokines and help in the resolution of inflammation (39). Recent studies in mice revealed that a combined deficiency of DNase 1 and DNase1L3 leads to a clotting of vessels by NETs in a disease resembling thrombotic microangiopathies (40).

Extra- and intra- cellular nucleases protect against inflammation and autoimmunity

If excessive nucleic acids are released by necrosis or the programmed pathways of necrosis discussed above, how do they re-enter the cell? One of the earliest re-entry mechanisms identified in SLE was through IgG autoantibodies that form immune complexes with nucleoprotein particles and engage the activating Fc gamma receptor, Fc γ RIIA on plasmacytoid dendritic cells (pDC) and other cells. This in turn led to phagocytosis and downstream activation of intracellular Toll Like Receptors (TLR) to stimulate the production of IFN-I and inflammatory cytokines (41). Other re-entry pathways include internalization facilitated by HMGB1 or the related mitochondrial protein, Tfam, through the RAGE receptor. There are less well defined mechanisms associated with endocytosis of MP,

possibly involving SCARF1 (scavenger receptor 1) and DNA in complex with the neutrophil peptide, LL37, binding to cell surface glycoproteins (42) (Fig. 3). It is relevant to note that the function of HMGB1 is influenced by its redox state: depending on the cysteine(s) oxidized, HMGB1 can activate TLR4, CXCL12 or be completely inactive (reviewed in (43). Since nucleic acids that have gained re-entry are now 'ectopic,' they are seen as foreign invaders and activate nucleic acid sensors (Fig. 3).

As emphasized above, inflammatory forms of cell death are associated with release of cellular contents that include DNA and RNA, into tissues and the bloodstream. Nucleic acids, whether derived from host or microbes are powerful DAMPs and PAMPs that elicit inflammation through multiple PRRs as detailed below. Extracellular nucleases such as DNase1, DNase1L3 and RNase1 provide the first line of protection by degrading the DNA and RNA that may be free or, more commonly, associated with proteins to form complexes (Fig. 3, Table 1). Cell surface associated chromatin on MP is degraded by a secreted DNase called DNase1L3 that contains a positively charged C-terminal peptide facilitating DNA digestion even when protein bound (44). There are many specialized nucleases within the cell, some of which are free in the cytosol such as DNase III (TREX1) whereas others are compartmentalized in endosomes (DNase II) (Table 1). TREX1 is an abundant 3–5' exonuclease with a preference for single stranded DNA and has been implicated in the degradation of DNA arising from retroelements and mitochondria, although digestion is less efficient if the DNA is oxidized (contains 8-OH deoxyguanine) (45). DNA:RNA hybrids that may be produced by an infection with retroviruses or occur in the life cycle of endogenous retroelements are degraded by RNaseH2 enzymes (Table 1). Mitochondrial DNA has recently received much attention as an agonist capable of stimulating IFN-I and other cytokines (34, 38). Since it is readily oxidized, mtDNA is less efficiently degraded by DNases such as TREX1 leading to activation of the DNA sensor, cGAS (34) (see below).

Disease relevance—In normal individuals, despite the fact that nucleic acids are partly shielded from nucleases by the proteins they are bound to, cell debris is cleared with no long-term consequences. 'Interferonopathies' are a group of monogenic orphan diseases affecting children that are characterized by an IFN-I gene expression signature in circulating blood (46). The best studied interferonopathy is Aicardi-Goutières Syndrome (AGS) (47), a disease characterized by abnormalities in the skin and brain resulting in severe neurologic defects with ~75% of patients being profoundly disabled in the first few years of life (48). The expression of lupus-like features including serology (49) in many of these patients is consistent with the idea that elevated levels of Type I IFNs other than IFN- α can also contribute to SLE pathogenesis. It is also relevant to point out that Type I IFN signatures are not only seen in SLE, but also in patients with Sjogren's syndrome, dermatomyositis and scleroderma.

Molecular genetic studies revealed that interferonopathies are produced by a number of mutations in genes that are involved in nucleic acid metabolism. Most relevant, in the disease Aicardi Goutières Syndrome (AGS), mutations in *TREX1*, *RNaseH*, *ADAR*, *IFIH1* activate pathways that stimulate the production of IFN- β (50, 51) (Table 1). Therefore, in diseases such as SLE and interferonopathies, the burden of nucleic acids exceeds the degradative capacity of either the extra- or intra-cellular nucleases because of increased

amount of dead cells and / or low nuclease enzyme levels. Rare cases of SLE are associated with loss of function mutations in DNase 1, DNase II or DNase1L3 and up to 2% of SLE patients have a heterozygous mutation in *TREX1* (DNase III) (52, 53) (Table I). As mentioned, oxidized DNA that is produced during NET formation, is less efficiently digested by TREX1 so may contribute to IFN-I stimulation (54).

Nucleic Acids stimulate Pattern Recognition Receptors to induce Inflammation

The location of nucleic acid sensing receptors can be conveniently divided into endosomal and cytosolic. Virus infections will often trigger both sets of receptors. Even in the case of ICs containing autoantigens with nuclear material, nucleic acids can leak from endosomal compartments into the cytosol (55, 56).

Endosomal sensors

TLRs can be divided into two categories, those that are mostly expressed on the cell surface (such as TLR2 and TLR4) and those expressed predominantly in the intracellular compartments, endosomes and lysosomes (Fig. 4). These include the RNA-sensing TLRs, TLR7 and TLR8 that detect ss-RNA and TLR3, that is more sensitive to ds-RNA whereas TLR9 senses double stranded DNA (dsDNA) (Fig. 4). Early studies of DNA suggested that bacterial DNA containing CpG was highly inflammatory whereas mammalian DNA that contained much less CpG was either inactive or suppressive. However, it is now accepted that, both the source of mammalian DNA (for example, mitochondrial DNA is relatively hypomethylated) and the proteins to which DNA binds, influence its inflammatory properties (Fig. 3). Because the endosomal TLRs are located within intracellular compartments, and not on the cell surface, one major factor that limits their activation is ligand accessibility—nucleic acids need to colocalize with the TLRs in the appropriate endosomal/lysosomal compartment in order to engage these receptors and signal cytokine responses. Access to intracellular TLR is also restricted by expression levels in different cell types frequently requiring a second signal such as FcγR2A activation for a cellular response (54). TLR7 and 9 are expressed mostly in pDC and B cells whereas TLR8 is highly expressed in human neutrophils. TLR3 is expressed by both hematopoietic and nonhematopoietic cells (e.g. fibroblasts). As discussed above, in addition to IFN-α, nucleic acid triggering of TLR7 or 9 induce pDCs to make pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL)-6. TLR3 activation also leads to the production of type I IFN and pro-inflammatory cytokines (Fig. 4).

Disease relevance—IFN-inducing activity in SLE and possibly other diseases such as Sjogren's Syndrome and Scleroderma can, in part, be explained by ICs consisting of autoantibodies bound to DNA- and RNA-associated autoantigens (57) being internalized by the FcγRII (CD32) receptor on pDCs and triggering the release of IFN-α (58). This basic observation has led to clinical trials evaluating biologics that degrade RNA in the immune complex, that target pDC or that block the IFN-I receptor for the treatment of SLE (59) (Fig. 5). Note that cell surface expressed TLRs, particularly TLR4, have been implicated in

rheumatic diseases such as RA and scleroderma where non-nucleic acid containing cell debris may act as ligands (see (60, 61).

Cytosolic Sensors

Following infection, many microbes replicate in the cytosol. To counter this, cells are replete with different sensors poised to respond to microbial nucleic acids by a variety of strategies that include immune activation (Fig. 4). Cytosolic RNA receptors, referred to as RLRs (RIG-I [retinoic acid-inducible gene I]-like receptors), comprise three DExD/H box RNA helicases, RIG-I, MDA-5 and LGP2. These receptors are widely expressed across tissues and cell types. The RLR proteins sense virus RNA in the cytoplasm of infected cells but differ in their specificities. RIG-I recognizes 5'-triphosphorylated, uncapped RNA as well as RNA bearing 5'-diphosphates. MDA5 recognizes long dsRNA and branched high-molecular RNA forms. Following activation, RIG-I and MDA5 bind to the mitochondrial-associated adaptor protein, MAVS (also known as IPS-1) that forms a scaffold for stimulation of IFN- β and other inflammatory cytokines by the pathways shown in Fig. 4. LGP2 may be a negative regulator of these processes.

At least 13 cytosolic DNA sensors have been identified (reviewed in (62). All of these DNA sensors stimulate the production of IFN-I or promote the assembly of an inflammasome complex, leading to caspase-1 activation and subsequent secretion of IL-1 β and IL-18 (Fig. 4). The AIM2-like receptors (ALR), of which there are 4 in humans and 13 in mice, have in common a DNA-binding HIN domain and a Pyrin signaling domain.(63), The biologic role for all of these DNA sensors *in vivo*, aside from Cyclic GMP-AMP synthase (cGAS), has been questioned (64) and a recent study suggests that cGAS-STING, rather than AIM2 is responsible for inflammasome activation in myeloid cells (65). We will therefore focus on the sensor, cGAS. Binding of dsDNA to cGAS causes a conformational change in the active site of cGAS, which utilizes ATP and GTP to synthesize the cyclic dinucleotide, 2'-5' cGAMP (66). cGAMP then functions as an endogenous second messenger binding directly to the adapter protein, Stimulator of Interferon Genes (STING). Dimerization of STING results in the translocation of STING from the ER to the Golgi with recruitment and activation of TBK1 and the transcription factor, IRF3 culminating in the synthesis of IFN- β (67) (Fig. 4).

Disease relevance—Key questions regarding immune stimulation in autoimmune and autoinflammatory diseases include: i) are the stimulatory nucleic acids derived from outside or inside the cell?; ii) which are the main sensors stimulated?; iii) what is the connection between nucleic acid immune activation and antinuclear antibody production?

In Interferonopathies, stimulatory nucleic acids almost certainly originate from within the cell due to defective nucleic acid metabolism and accumulation of nucleic acids resulting in stimulation of the cytosolic sensors. In SLE and related disorders, extracellular origins such as ICs, MP and extruded nuclei can activate both TLR and cytosolic sensors once internalized. Intriguingly, recent studies have implicated retroelement-derived nucleic acids in immune activation in SLE and Sjogren's Syndrome. The SINE (short interspersed nuclear element), Alu, was detected in IC in SLE (68) and the long interspersed nuclear element,

LINE-1 was detected in the kidneys and salivary glands of Sjogren's and SLE patients respectively (69). Could cell intrinsic activation stimulate and prime for extrinsic (particularly IC) stimulation? Could both endosomal and cytosolic sensors be involved at different phases of disease?

Given the pivotal role of cGAS-STING in the generation of IFN-I and central role played by IFN-I in the pathogenesis of SLE, cGAS expression and cGAMP production were investigated in SLE patients (70). There was an increase in cGAS transcript expression in ~1/3 of SLE patients and ~15% of patients had detectable cGAMP dinucleotide in peripheral blood cells (PBMC). Interestingly, the patients with detectable cGAMP had a higher SLEDAI score than patients without activation of the pathway (70). With regard to RNA sensors, aggregation of MAVS has been demonstrated in patients with SLE which may be associated with oxidation of mtDNA (71, 72). Hasan et al (73) reported that TBK1 (a protein downstream of cGAS as well as RLR) mRNA expression was significantly increased in SLE patients, especially in monocytes. Finally, several patients with gain of function mutations in the adapter, STING, developed the auto-inflammatory / autoimmune diseases SAVI and chilblain lupus (74, 75). Very likely, the phenotypic expression of disease is influenced by a multitude of other gene variants and environmental factors.

The hallmark of systemic autoimmune diseases is the presence of 'antinuclear antibodies' (ANA). Considering the importance nucleic acids discussed in this review, the question arises as to whether excessive exposure to, or defective removal / degradation of, nucleic acids is sufficient to stimulate the production of ANA? The answer is provided by studies of patients with the interferonopathy, Aicardi Goutières Syndrome (AGS), where highly statistically significant increases in autoantibodies to Sm/RNP and to SSA/SSB were observed (49). Considering that nucleic acid stimulation of cytokines is cell intrinsic in AGS (Fig. 3), this suggests that cytokines, especially IFN-I can be sufficient to stimulate ANA generation but these findings do not exclude other stimuli such as B cell receptor engagement by cell debris or, in the case of DNase1L3 deficiency, stimulation by microparticles (44). Presumably, IFN acts by its adjuvant effect leading to activation of all types of immune cell (76). Since most patients with monogenic interferonopathies generate autoantibodies but do not develop full blown SLE, other genetic variants, for example in *PTPN22*, *BLK*, *BANK1* that influence B cell function, are likely required for the expression of high affinity antibodies that cause systemic disease such as glomerulonephritis.

How are self and non self DNA/RNA distinguished?

The Special Case of Mitochondria – is 1.5 billion years not enough?

Mitochondria are self-contained organelles that evolved from a protokaryote co-opted into eukaryotic cells ~1.5 billion years ago. They perform vital functions within the cell. They produce high efficiency energy by generating ATP through oxidative phosphorylation and mitochondrial proteins such as Bcl-2 and Bax/Bak control cell survival and death by regulating mitochondrial membrane potential. Mitochondria are readily damaged through hyper- or hypo- polarization of their membranes as well as by generation of reactive oxygen species (ROS). Damaged mitochondria are usually removed by the process of mitophagy but neutrophils appear to be poor at mitophagy and therefore extrude damaged mitochondria as

a way to prevent untoward damage within the cell (34, 38). Mitochondria therefore impact immunity because: their DNA is rich in CpG DNA (potential TLR9 ligand) and can gain entry into cells through binding to Tfam (77); ROS oxidizes mtDNA (potential cGAS ligand) that cannot be efficiently repaired within the organelle; in mammalian cells they are the only source of cardiolipin (antigenic target of some autoantibodies); and the damaged organelle can be extruded (making it accessible to the immune system). Furthermore, like their ancient bacterial forebears, mitochondrial peptides have a N-terminal formylated methionine that stimulate the production of chemokines. All considered, while a necessity for the eukaryotic lifestyle, mitochondria pose an outsize danger to innate immunity (78).

Modified Nucleic Acids

It took decades to determine how cells of the adaptive immune system distinguish self from foreign antigens and research is still ongoing. Although some of the mechanisms are ancient (for example cGAS-STING is estimated to have evolved 600 million years ago), our understanding of the rules for distinguishing self from foreign by innate immune sensors is at an early stage. As discussed, self –nonself distinction in innate immunity relies on non-discriminatory strategies that include degradation of extracellular nucleic acids (mostly from self), limited cell type expression of some sensors and intracellular location of all known nucleic acid sensors. Discriminatory strategies that identify differences between self and foreign nucleic acids by sequence, chemical modifications or structure are being discovered. For RNA, 5' capping of the terminus is an excellent example, as this feature is present in many viruses, but not host RNA (79). RNA-reactive TLRs can detect both virus and mammalian RNA but here again, sequence and structure are most likely key determinants of ligand avidity, as TLR7 and TLR8 preferentially bind unmodified uridine (U)-rich ssRNAs (80, 81). Many of the small RNAs associated with their protein antigens (e.g. Sm/RNP, Ro/La) in SLE fit this category (poly-U rich RNAs). With regard to DNA, oxidation of DNA and CpG methylation demonstrate selectivity of processing and recognition as discussed. Whereas DNase1 can degrade oxidized DNA, oxidation impairs activity of the intracellular DNase, Trex1, resulting in the increased likelihood of stimulation of cGAS (45). TLR9 is the main sensor of DNA and was originally thought to distinguish microbial DNA from mammalian DNA on the basis of reactivity with hypomethylated CpG motifs, which are less commonly found in mammalian DNA but are common in bacterial and viral DNA. TLR9 can also detect mammalian DNA. Nevertheless, DNA sequence is still relevant because mammalian DNA sequences enriched for CpG dinucleotides are more potent activators of TLR9 than DNA sequences devoid of CpG dinucleotides (82). Potential sources of immunostimulatory mammalian DNA include CpG islands, mitochondrial DNA, and retroelements (Fig. 3).

Concluding Remarks and Therapeutic Opportunities

A deeper understanding of the origin of immunostimulatory nucleic acids and their consequences enables a broad approach to treating diseases associated with release of cell debris. As shown in Fig. 5, specific targets can, and in some cases have already been used to mitigate the inflammatory effects of nucleic acids or nucleoprotein complexes. In widespread use are biologics to block cytokines (#2) and newer small molecule drugs such

as ‘Jak inhibitors’ are being introduced to attenuate their downstream effects (#1 in Fig. 5). Cell depletion studies (T or B lymphocytes) have largely failed, but pDC (#3) represent an attractive target in IFN-I related diseases. Drug strategies to target the intracellular sensors themselves are a logical approach but inhibitory oligonucleotides to block TLR (#4), although effective in vitro, have not emerged as a viable therapy for patients. Two potentially relevant targets downstream of TLR are IRAK4 and IRF5 and efforts are underway to block the function of these proteins (83). Interestingly, antimalarial drugs were shown to inhibit cGAS (#5) in vitro and modified forms are now being tested in preclinical models of disease such as AGS (84). In general, strategies to inhibit the most upstream pathways are desirable since they are least likely to limit inflammatory responses to microbes in host defense. Chemical inhibitors are more likely to have off target effects compared to biologics. Further understanding of cell death and intracellular nucleic acid metabolism will undoubtedly help refine our therapeutic approach to treat patients with a wide range of autoinflammatory and autoimmune disorders.

Acknowledgments

Financial support: Dr. Elkon’s work is supported by the National Institutes of Health and the Lupus Research Alliance.

The author thanks Sladjana Skopelja-Gardner and other colleagues at the University of Washington for comments and discussion.

References

1. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature*. 1992; 356:314–7. [PubMed: 1372394]
2. Casciola-Rosen LA, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *Journal of Experimental Medicine*. 1994; 179:1317–30. [PubMed: 7511686]
3. Martin SJ, Henry CM, Cullen SP. A perspective on mammalian caspases as positive and negative regulators of inflammation. *Mol Cell*. 2012; 46(4):387–97. [PubMed: 22633487]
4. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol*. 1989; 54(Pt 1):1–13.
5. Matzinger P. The danger model: a renewed sense of self. *Science*. 2002; 296(5566):301–5. [PubMed: 11951032]
6. Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat Rev Mol Cell Biol*. 2010; 11(10):700–14. [PubMed: 20823910]
7. Shaw TJ, Martin P. Wound repair: a showcase for cell plasticity and migration. *Curr Opin Cell Biol*. 2016; 42:29–37. [PubMed: 27085790]
8. Pisetsky DS, Gauley J, Ullal AJ. Microparticles as a source of extracellular DNA. *Immunologic research*. 2011; 49(1–3):227–34. [PubMed: 21132466]
9. Robbins PD, Dorronsoro A, Booker CN. Regulation of chronic inflammatory and immune processes by extracellular vesicles. *The Journal of clinical investigation*. 2016; 126(4):1173–80. [PubMed: 27035808]
10. Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. *Cell*. 2010; 140(5):619–30. [PubMed: 20211132]
11. Baumann I, Kolowos W, Voll RE, Manger B, Gaipl U, Neuhuber WL, et al. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. *Arthritis and rheumatism*. 2002; 46(1):191–201. [PubMed: 11817590]

12. Mevorach D, Mascarenhas J, Gershov DA, Elkon KB. Complement-dependent clearance of apoptotic cells by human macrophages. *J Exp Med*. 1998; 188:2313–20. [PubMed: 9858517]
13. Botto M, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, et al. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nature genetics*. 1998; 19(1):56–9. [PubMed: 9590289]
14. Manderson AP, Botto M, Walport MJ. The Role of Complement in the Development of Systemic Lupus Erythematosus. *Annu Rev Immunol*. 2004; 22:431–56. [PubMed: 15032584]
15. Gershov D, Kim S, Brot N, Elkon KB. C-Reactive Protein Binds to Apoptotic Cells, Protects the Cells from Assembly of the Terminal Complement Components, and Sustains an Antiinflammatory Innate Immune Response. Implications for systemic autoimmunity. *The Journal of experimental medicine*. 2000; 192(9):1353–64. [PubMed: 11067883]
16. Cookson BT, Brennan MA. Pro-inflammatory programmed cell death. *Trends Microbiol*. 2001; 9(3):113–4. [PubMed: 11303500]
17. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell*. 2002; 10(2):417–26. [PubMed: 12191486]
18. Franchi L, Munoz-Planillo R, Nunez G. Sensing and reacting to microbes through the inflammasomes. *Nat Immunol*. 2012; 13(4):325–32. [PubMed: 22430785]
19. Sansonetti PJ, Phalipon A, Arondel J, Thirumalai K, Banerjee S, Akira S, et al. Caspase-1 activation of IL-1beta and IL-18 are essential for Shigella flexneri-induced inflammation. *Immunity*. 2000; 12(5):581–90. [PubMed: 10843390]
20. Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, Warming S, et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature*. 2015; 526(7575):666–71. [PubMed: 26375259]
21. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*. 2015; 526(7575):660–5. [PubMed: 26375003]
22. Rock KL, Latz E, Ontiveros F, Kono H. The sterile inflammatory response. *Annu Rev Immunol*. 2010; 28:321–42. [PubMed: 20307211]
23. Dorfleutner A, Stehlik C. A dRAStic RHOAdblock of Pyrin inflammasome activation. *Nat Immunol*. 2016; 17(8):900–2. [PubMed: 27434003]
24. Pascart T, Richette P. Current and future therapies for gout. *Expert opinion on pharmacotherapy*. 2017; 18(12):1201–11. [PubMed: 28689430]
25. Linkermann A, Green DR. Necroptosis. *N Engl J Med*. 2014; 370(5):455–65. [PubMed: 24476434]
26. Sun L, Wang H, Wang Z, He S, Chen S, Liao D, et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell*. 2012; 148(1–2):213–27. [PubMed: 22265413]
27. Weinlich R, Oberst A, Beere HM, Green DR. Necroptosis in development, inflammation and disease. *Nat Rev Mol Cell Biol*. 2017; 18(2):127–36. [PubMed: 27999438]
28. Zhu L, Yang X, Chen W, Li X, Ji Y, Mao H, et al. Decreased expressions of the TNF-alpha signaling adapters in peripheral blood mononuclear cells (PBMCs) are correlated with disease activity in patients with systemic lupus erythematosus. *Clinical rheumatology*. 2007; 26(9):1481–9. [PubMed: 17235653]
29. Lee SH, Kwon JY, Kim SY, Jung K, Cho ML. Interferon-gamma regulates inflammatory cell death by targeting necroptosis in experimental autoimmune arthritis. *Scientific reports*. 2017; 7(1): 10133. [PubMed: 28860618]
30. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004; 303(5663):1532–5. [PubMed: 15001782]
31. Lee KH, Kronbichler A, Park DD, Park Y, Moon H, Kim H, et al. Neutrophil extracellular traps (NETs) in autoimmune diseases: A comprehensive review. *Autoimmunity reviews*. 2017; 16(11): 1160–73. [PubMed: 28899799]
32. Gupta S, Kaplan MJ. The role of neutrophils and NETosis in autoimmune and renal diseases. *Nature reviews Nephrology*. 2016; 12(7):402–13. [PubMed: 27241241]

33. Hakkim A, Furnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(21):9813–8. [PubMed: 20439745]
34. Lood C, Blanco LP, Purmalek MM, Carmona-Rivera C, De Ravin SS, Smith CK, et al. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nature medicine*. 2016
35. Knight JS, Zhao W, Luo W, Subramanian V, O'Dell AA, Yalavarthi S, et al. Peptidylarginine deiminase inhibition is immunomodulatory and vasculoprotective in murine lupus. *The Journal of clinical investigation*. 2013; 123(7):2981–93. [PubMed: 23722903]
36. Campbell AM, Kashgarian M, Shlomchik MJ. NADPH oxidase inhibits the pathogenesis of systemic lupus erythematosus. *Science translational medicine*. 2012; 4(157):157ra41.
37. Gordon RA, Herter JM, Rosetti F, Campbell AM, Nishi H, Kashgarian M, et al. Lupus and proliferative nephritis are PAD4 independent in murine models. *JCI insight*. 2017; 2(10)
38. Caielli S, Athale S, Domic B, Murat E, Chandra M, Banchereau R, et al. Oxidized mitochondrial nucleoids released by neutrophils drive type I interferon production in human lupus. *The Journal of experimental medicine*. 2016; 213(5):697–713. [PubMed: 27091841]
39. Schauer C, Janko C, Munoz LE, Zhao Y, Kienhofer D, Frey B, et al. Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. *Nature medicine*. 2014; 20(5):511–7.
40. Jimenez-Alcazar M, Rangaswamy C, Panda R, Bitterling J, Simsek YJ, Long AT, et al. Host DNases prevent vascular occlusion by neutrophil extracellular traps. *Science*. 2017; 358(6367):1202–6. [PubMed: 29191910]
41. Bave U, Vallin H, Alm GV, Ronnblom L. Activation of natural interferon-alpha producing cells by apoptotic U937 cells combined with lupus IgG and its regulation by cytokines. *Journal of autoimmunity*. 2001; 17(1):71–80. [PubMed: 11488639]
42. Chamilos G, Gregorio J, Meller S, Lande R, Kontoyiannis DP, Modlin RL, et al. Cytosolic sensing of extracellular self-DNA transported into monocytes by the antimicrobial peptide LL37. *Blood*. 2012; 120(18):3699–707. [PubMed: 22927244]
43. Pisetsky DS. The translocation of nuclear molecules during inflammation and cell death. *Antioxid Redox Signal*. 2014; 20(7):1117–25. [PubMed: 23373769]
44. Sisirak V, Sally B, D'Agati V, Martinez-Ortiz W, Ozcakar ZB, David J, et al. Digestion of Chromatin in Apoptotic Cell Microparticles Prevents Autoimmunity. *Cell*. 2016; 166(1):88–101. [PubMed: 27293190]
45. Gehrke N, Mertens C, Zillinger T, Wenzel J, Bald T, Zahn S, et al. Oxidative Damage of DNA Confers Resistance to Cytosolic Nuclease TREX1 Degradation and Potentiates STING-Dependent Immune Sensing. *Immunity*. 2013; 39(3):482–95. [PubMed: 23993650]
46. Crow YJ, Manel N. Aicardi-Goutieres syndrome and the type I interferonopathies. *Nature reviews Immunology*. 2015; 15(7):429–40.
47. Aicardi J, Goutieres F. A progressive familial encephalopathy in infancy with calcifications of the basal ganglia and chronic cerebrospinal fluid lymphocytosis. *Ann Neurol*. 1984; 15(1):49–54. [PubMed: 6712192]
48. Crow YJ, Chase DS, Lowenstein Schmidt J, Szykiewicz M, Forte GM, Gornall HL, et al. Characterization of human disease phenotypes associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, and IFIH1. *Am J Med Genet A*. 2015; 167A(2):296–312. [PubMed: 25604658]
49. Cuadrado E, Vanderver A, Brown KJ, Sandza A, Takanohashi A, Jansen MH, et al. Aicardi-Goutieres syndrome harbours abundant systemic and brain-reactive autoantibodies. *Annals of the rheumatic diseases*. 2014
50. Crow YJ. Type I interferonopathies: mendelian type I interferon up-regulation. *Curr Opin Immunol*. 2015; 32:7–12. [PubMed: 25463593]
51. Rice GI, del Toro Duany Y, Jenkinson EM, Forte GM, Anderson BH, Ariaudo G, et al. Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling. *Nature genetics*. 2014; 46(5):503–9. [PubMed: 24686847]

52. Lee-Kirsch MA, Gong M, Chowdhury D, Senenko L, Engel K, Lee YA, et al. Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *Nature genetics*. 2007; 39(9):1065–7. [PubMed: 17660818]
53. Namjou B, Kothari PH, Kelly JA, Glenn SB, Ojwang JO, Adler A, et al. Evaluation of the TREX1 gene in a large multi-ancestral lupus cohort. *Genes Immun*. 2011; 12(4):270–9. [PubMed: 21270825]
54. Lood C, Arve S, Ledbetter J, Elkon KB. TLR7/8 activation in neutrophils impairs immune complex phagocytosis through shedding of FcγRIIA. *The Journal of experimental medicine*. 2017; 214(7):2103–19. [PubMed: 28606989]
55. Monteith AJ, Kang S, Scott E, Hillman K, Rajfur Z, Jacobson K, et al. Defects in lysosomal maturation facilitate the activation of innate sensors in systemic lupus erythematosus. *Proceedings of the National Academy of Sciences of the United States of America*. 2016; 113(15):E2142–51. [PubMed: 27035940]
56. Ahn J, Gutman D, Saijo S, Barber GN. STING manifests self DNA-dependent inflammatory disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109(47):19386–91. [PubMed: 23132945]
57. Doedens JR, Jones WD, Hill K, Mason MJ, Gersuk VH, Mease PJ, et al. Blood-Borne RNA Correlates with Disease Activity and IFN-Stimulated Gene Expression in Systemic Lupus Erythematosus. *Journal of immunology*. 2016; 197(7):2854–63.
58. Bave U, Magnusson M, Eloranta ML, Perers A, Alm GV, Ronnblom L. Fc gamma RIIa is expressed on natural IFN-α-producing cells (plasmacytoid dendritic cells) and is required for the IFN-α production induced by apoptotic cells combined with lupus IgG. *Journal of immunology*. 2003; 171(6):3296–302.
59. Furie R, Khamashta M, Merrill JT, Werth VP, Kalunian K, Brohawn P, et al. Anifrolumab, an Anti-Interferon-α Receptor Monoclonal Antibody, in Moderate-to-Severe Systemic Lupus Erythematosus. *Arthritis & rheumatology*. 2017; 69(2):376–86. [PubMed: 28130918]
60. Bhattacharyya S, Varga J. *Immunology letters*. 2017. Endogenous ligands of TLR4 promote unresolving tissue fibrosis: Implications for systemic sclerosis and its targeted therapy.
61. Elshabrawy HA, Essani AE, Szekanecz Z, Fox DA, Shahrara S. TLRs, future potential therapeutic targets for RA. *Autoimmunity reviews*. 2017; 16(2):103–13. [PubMed: 27988432]
62. Dempsey A, Bowie AG. Innate immune recognition of DNA: A recent history. *Virology*. 2015; 479–480:146–52.
63. Brunette RL, Young JM, Whitley DG, Brodsky IE, Malik HS, Stetson DB. Extensive evolutionary and functional diversity among mammalian AIM2-like receptors. *The Journal of experimental medicine*. 2012; 209(11):1969–83. [PubMed: 23045604]
64. Vance RE. Cytosolic DNA Sensing: The Field Narrows. *Immunity*. 2016; 45(2):227–8. [PubMed: 27533006]
65. Gaidt MM, Ebert TS, Chauhan D, Ramshorn K, Pinci F, Zuber S, et al. The DNA Inflammasome in Human Myeloid Cells Is Initiated by a STING-Cell Death Program Upstream of NLRP3. *Cell*. 2017; 171(5):1110–24. e18. [PubMed: 29033128]
66. Gao D, Wu J, Wu YT, Du F, Aroh C, Yan N, et al. Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. *Science*. 2013; 341(6148):903–6. [PubMed: 23929945]
67. Cai X, Chiu YH, Chen ZJ. The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. *Mol Cell*. 2014; 54(2):289–96. [PubMed: 24766893]
68. Hung T, Pratt GA, Sundararaman B, Townsend MJ, Chaivorapol C, Bhangale T, et al. The Ro60 autoantigen binds endogenous retroelements and regulates inflammatory gene expression. *Science*. 2015; 350(6259):455–9. [PubMed: 26382853]
69. Mavragani CP, Sagalovskiy I, Guo Q, Nezos A, Kapsogeorgou EK, Lu P, et al. Expression of Long Interspersed Nuclear Element 1 Retroelements and Induction of Type I Interferon in Patients With Systemic Autoimmune Disease. *Arthritis & rheumatology*. 2016; 68(11):2686–96. [PubMed: 27338297]
70. An J, Durcan L, Woodward JJ, Karr R, Teale T, Elkon KB. cGAMP and cGAS are Expressed in a Subset of Patients with Systemic Lupus Erythematosus and Associate with Disease Activity. *Arthritis and rheumatism*. 2017; 69:800–7.

71. Shao WH, Shu DH, Zhen Y, Hilliard B, Priest SO, Cesaroni M, et al. Prion-like Aggregation of Mitochondrial Antiviral Signaling Protein in Lupus Patients Is Associated With Increased Levels of Type I Interferon. *Arthritis & rheumatology*. 2016; 68(11):2697–707. [PubMed: 27110677]
72. Buskiewicz IA, Montgomery T, Yasewicz EC, Huber SA, Murphy MP, Hartley RC, et al. Reactive oxygen species induce virus-independent MAVS oligomerization in systemic lupus erythematosus. *Sci Signal*. 2016; 9(456):ra115. [PubMed: 27899525]
73. Hasan M, Dobbs N, Khan S, White MA, Wakeland EK, Li QZ, et al. Cutting Edge: Inhibiting TBK1 by Compound II Ameliorates Autoimmune Disease in Mice. *Journal of immunology*. 2015; 195(10):4573–7.
74. Konig N, Fiehn C, Wolf C, Schuster M, Cura Costa E, Tungler V, et al. Familial chilblain lupus due to a gain-of-function mutation in STING. *Annals of the rheumatic diseases*. 2017; 76(2):468–72. [PubMed: 27566796]
75. Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Montealegre Sanchez GA, et al. Activated STING in a vascular and pulmonary syndrome. *N Engl J Med*. 2014; 371(6):507–18. [PubMed: 25029335]
76. Ronnblom L, Elkon KB. Cytokines as therapeutic targets in SLE. *Nature reviews Rheumatology*. 2010; 6(6):339–47. [PubMed: 20440285]
77. Julian MW, Shao G, Bao S, Knoell DL, Papenfuss TL, VanGundy ZC, et al. Mitochondrial transcription factor A serves as a danger signal by augmenting plasmacytoid dendritic cell responses to DNA. *Journal of immunology*. 2012; 189(1):433–43.
78. Rongvaux A. Innate immunity and tolerance toward mitochondria. *Mitochondrion*. 2017
79. Goubau D, Schlee M, Deddouche S, Pruijssers AJ, Zillinger T, Goldeck M, et al. Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5'-diphosphates. *Nature*. 2014; 514(7522):372–5. [PubMed: 25119032]
80. Lau CM, Broughton C, Tabor AS, Akira S, Flavel RA, Mamula MJ, et al. RNA-associated autoantigens activate B cells by combined B cell receptor/Toll-like receptor 7 engagement. *The Journal of experimental medicine*. 2005; 202:1171–7. [PubMed: 16260486]
81. Vollmer J, Tluk S, Schmitz C, Hamm S, Jurk M, Forsbach A, et al. Immune stimulation mediated by autoantigen binding sites within small nuclear RNAs involves Toll-like receptors 7 and 8. *The Journal of experimental medicine*. 2005; 202(202):1575–85. [PubMed: 16330816]
82. Uccellini MB, Busconi L, Green NM, Busto P, Christensen SR, Shlomchik MJ, et al. Autoreactive B cells discriminate CpG-rich and CpG-poor DNA and this response is modulated by IFN- α . *Journal of immunology*. 2008; 181(9):5875–84.
83. Genung NE, Guckian KM. Small Molecule Inhibition of Interleukin-1 Receptor-Associated Kinase 4 (IRAK4). *Progress in medicinal chemistry*. 2017; 56:117–63. [PubMed: 28314411]
84. An J, Woodward JJ, Minie M, Sasaki T, Elkon KB. Novel Anti-Malarial Drug Derivative Inhibited Type I Interferon Production and Autoimmune Inflammation through Inhibition of CGAS-Sting Pathway in *Trex1*^{-/-} Mouse. *Arthritis & rheumatology*. 2016; 68(Supplement S10) Abstract 2984.
85. Yasutomo K, Horiuchi T, Kagami S, Tsukamoto H, Hashimura C, Urushihara M, et al. Mutation of DNASE1 in people with systemic lupus erythematosus. *Nature genetics*. 2001; 28(4):313–4. [PubMed: 11479590]
86. Rodero MP, Tesser A, Bartok E, Rice GI, Della Mina E, Depp M, et al. Type I interferon-mediated autoinflammation due to DNase II deficiency. *Nature communications*. 2017; 8(1):2176.
87. Kawane K, Ohtani M, Miwa K, Kizawa T, Kanbara Y, Yoshioka Y, et al. Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. *Nature*. 2006; 443(7114):998–1002. [PubMed: 17066036]
88. Stam AH, Kothari PH, Shaikh A, Gschwendter A, Jen JC, Hodgkinson S, et al. Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations. *Brain : a journal of neurology*. 2016; 139(11):2909–22. [PubMed: 27604306]
89. Al-Mayouf SM, Sunker A, Abdwani R, Abrawi SA, Almurshedi F, Alhashmi N, et al. Loss-of-function variant in DNASE1L3 causes a familial form of systemic lupus erythematosus. *Nature genetics*. 2011; 43(12):1186–8. [PubMed: 22019780]

Figure 1. Basic concepts of Innate cell stimulation by Damage (DAMPs) or Pathogen (PAMPs) Associated Molecular Patterns. Either DAMPs or PAMPs are capable of activating cells of the innate immune system such as macrophages and dendritic cells. Depending on the nature of molecular pattern, either anti- or pro-inflammatory responses can be elicited. These responses impact both the inflammatory environment as well as communication with T cells as shown in the figure. Whereas intact apoptotic cells are immunologically silent or stimulate anti-inflammatory responses as well as negative adaptive responses (as in the left panel), release of cellular content from necrotic cells is almost always inflammatory (as in the right panel). Note that DAMPs and PAMPs also impact B cells both by serving as antigens activating the B cell receptor and, following internalization, by activation of sensors such as TLR 7 and 9 (see also Fig. 3).

Figure 2. Immediate or Programmed forms of necrosis stimulate inflammation

Programmed forms of necrosis include pyroptosis, necroptosis and NET formation – see text for details. Common features are damage to the cell membrane and release of cytokines, although this is less well established for NET formation. Gasdermin-D (GSDMD) and mixed lineage kinase-domain like (MLKL) are proteins involved in pore formation in pyroptosis and necroptosis respectively. How chromatin escapes from neutrophils during NET formation is unknown.

Figure 3. Nucleic acids gain access to intracellular sensors through multiple routes

Extracellular nucleic acids (DNA or RNA) can be captured by autoantibodies, bind to highly cationic peptides such as LL-37 or interact with nucleic acid binding proteins such as HMGB1 to gain re-entry into cells. A professional phagocytic cell such as the macrophage depicted on the right may uptake molecules by all of these pathways, whereas in other cell types, uptake may be more restricted. Endocytosis of nucleic acid complexes may be receptor mediated (Fcγ receptor and RAGE receptors are shown) but in other cases, mechanisms are less clear as discussed in the text. Nucleic acid complexes that enter endosomes generally trigger TLRs but escape into the cytosol has been documented in many situations (green arrow). Intracellular nucleic acids gain access to sensors through different mechanisms – mitochondrial DNA may be released directly from the damaged organelle or reenter the cell after binding to the protein called Tfam. The cytosolic sensors can be activated by DNA:RNA hybrids as well as nuclear DNA arising from retroelements or nuclear damage. Note that cell debris, especially highly charged DNA and RNA may also engage and be internalized by the B cell receptor thereby delivering to the B cell signals through BCR as well as TLR.

Figure 4. Sensors of intracellular nucleic acids

Intracellular receptors responsive to DNA (black) and RNA (red) are shown. The subset of nucleic acid responsive TLRs are present in the endosomal compartment whereas most other sensors are present in the cytosol. The signal transduction and also the transcription pathways are simplified. Adapters / platforms are shown in blue, red arrows indicate phosphorylation reactions; transcription factors are shaded in yellow. **Abbreviations:** AIM2, absent in melanoma-2; ALRs, AIM2-like receptors;; cGAMP, cyclic GMP–AMP; cGAS, cGAMP synthase; ER, endoplasmic reticulum; IFN, interferon; IKK (IKB kinase); IL, interleukin; IRAK (interleukin-1 receptor-associated kinase 4), IRF, IFN regulatory factor; MyD88, myeloid differentiation primary response protein-88; NALP3, **NACHT, LRR and PYD domains-containing protein 3**; PRR, pattern recognition receptor; RIG-I, retinoic acid–inducible gene I; STING, stimulator of IFN genes; TBK (TANK binding kinase); TLR, Toll-like receptor.

Figure 5. Potential points of therapeutic intervention in response to nucleic acid stimulation of sensors

Biologic or small molecule drugs have been used in patients or in preclinical models to block cytokines (#2) or downstream signaling events (#1). Antibodies targeting plasmacytoid dendritic cells are in early clinical trials (#3). The intracellular TLR sensors can be inhibited directly by oligonucleotides (ODN) or TLR signaling can be modulated by chemical inhibitors of IRF5 or IRAK4 (#4). Similarly, cGAS-STING activation or signaling can be attenuated by antimalarial drugs or TBK1 inhibitors (#5). Nucleases like DNase and RNase (#6) can degrade nucleic acid antigens that are free, in immune complexes or released by NET formation. Inhibitors of PAD4 or mitochondrial ROS inhibit the process of NET formation (#7). See text for details.

Table 1

Nucleases associated with autoimmune and autoinflammatory diseases

Nuclease	Location	Substrate(s)	* Disease Associations	References
DNase I	Blood, Intestine	ss and ds DNA	SLE ** Thrombotic microangiopathies	(85) (40)
DNase II	Endosome	DNA, nuclear fragments	SLE-like disease ** Arthritis	(86, 87)
DNase III (TREX1)	Cytosol	ss and ds DNA	AGS, RVCL SLE	(50, 88)
DNase 1L3	Blood	DNA in chromatin	SLE, HUVS	(89)
RNase H2	Cytosol	RNA:DNA hybrid DNA with misincorporated ribonucleotides	AGS	(50)

* All disease in humans, unless marked by ** indicating shown only in mice. Note that NET associated thrombotic microangiopathy occurs only with combined deficiency of DNase1 and DNase1L3.

Abbreviations: AGS, Aicardi Goutiere's Syndrome; ds, double stranded; HUVS, hypocomplementemic urticarial vasculitis ss, single stranded; SLE, Systemic lupus erythematosus; RVCL, Retinal Vasculopathy with Cerebral Leukoencephalopathy.