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The Origin and Properties of Extracellular DNA: From PAMP to DAMP

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Abstract

DNA is a polymeric macromolecule whose biological activities depend on location as well as binding to associated molecules. Inside the cell, DNA is the source of genetic information and binds histones to form nucleosomes. DNA can exit the cell, however, to enter the extracellular space primarily during cell death, either apoptosis or necrosis, as well as NETosis. While bacterial DNA is a potent immune stimulant by virtue of its CpG motifs, mammalian DNA, which is ordinarily inactive, can acquire activity by associating with nuclear, cytoplasmic and serum proteins which promote its uptake into cells to stimulate internal DNA sensors, including toll-like receptor 9. Among these proteins, anti-DNA autoantibodies can form immune complexes with DNA to stimulate plasmacytoid dendritic cells to produce type 1 interferon. Together, these findings suggest that the immune properties of DNA are mutable and diverse, reflecting its context and the array of attached molecules.

Keywords

DNA; anti-DNA; systemic lupus erythematosus; apoptosis; immune complexes; HMGB1

1. Introduction

DNA is polymeric macromolecule whose immunological activities can impact on normal as well as aberrant immunity. In the context of immunity, DNA displays three features important for its function: backbone structure, base sequence and intermolecular interaction. Depending on the interplay of these elements, DNA can be essentially inert, on the one hand, or powerfully active, on the other [1]. In addition to driving responses by itself, DNA can bind other molecules to form complexes whose activities exceed those of the individual components and create unique “danger” signals. Danger denotes damage or injury to the organism that triggers an immune response. The potential role of DNA in host defense is

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⁵Conflict of Interest Statement

The author declares that there is no conflict of interest.

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illustrated by the abundance and ubiquity of cellular DNA sensors that include both Toll-like receptors (TLR) and non-TLR receptors [2-6].

For DNA to stimulate immunity, it must access internal sensors, an event that can occur with either DNA located on the inside of the cell or with DNA that has entered the cell from the outside. Intracellular DNA most likely arises during infection with intracellular organisms, especially viruses, which replicate in the cytoplasm [3-6]. Because it originates during replication, this DNA may have fewer attached proteins and therefore may be more accessible for interaction with nucleic acid sensors. In contrast, extracellular DNA of host origin emerges from nucleated cells dying by apoptosis, necrosis or NETosis. NETosis denotes a unique response, primarily of neutrophils, in which nuclear DNA, following mixing with cytoplasmic contents, emerges from the cell as a NET (neutrophil extracellular trap) [7-9]. Once DNA has left a cell by any of these mechanisms, it can re-enter another cell especially when bound to antibodies or nucleic acid-binding proteins that provide a vehicle for intracellular uptake.

This review will consider the origin and structure of extracellular DNA, its immune properties and its potential as a target for new therapy. Key to this discussion is the idea that, while DNA can be measured biochemically as an isolated molecule, the immune properties are mutable since DNA most likely functions in complexes with other molecules which, as an ensemble, can signal danger.

2. Immune Properties of Extracellular DNA

2.1 Structure of DNA

The first element of DNA structure is the backbone. As shown initially by Watson and Crick, DNA can display a helical structure in which two chains intertwine to form a left-handed double helix which is very durable and stable. The second element of structure is the base sequence which conveys information for coding proteins and gene regulation. Sequence is highly variable among species and, furthermore, is subject to epigenetic modification by base methylation reactions which can alter the interaction of DNA with proteins [1].

The third element of DNA structure important in immunity relates to protein binding. In chromatin inside the cell nucleus, DNA is wrapped around a histone core comprised of two copies each of histone H2A, H2B, H3 and H4; the DNA between nucleosomes is termed linker DNA [10]. The structure of chromatin is not static, however, and other proteins can bind DNA, including inter-nucleosomal stretches. Among these proteins, histone H1 and HMGB1, a non-histone nuclear protein, occur abundantly in the nucleus and have more transient and less stable DNA binding than that of the core histones. By its nature, DNA is made for protein interaction and exposure of DNA in a free or “naked” form in either the intracellular or extracellular space may be limited at best.

Since DNA is a source of genetic information, an intact structure is essential for cell survival and division, a possibility assured by systems that recognize and repair DNA damage. Nevertheless, as part of apoptosis, a form of programmed cell death, DNA is subject to extensive digestion mediated by cellular DNases [11-14]. These reactions can cleave DNA to generate lower molecular weight DNA, including “ladders,” in which DNA exists in multiples of the usual length of DNA that surrounds the core histones. The function of cleavage reactions during apoptosis is not known although they may represent a defense system to degrade and inactivate DNA from infecting organisms such as viruses. Cleavage of DNA may also facilitate the phagocytic clearance of debris from apoptotic cells; any DNA that escapes phagocytic clearance may be eliminated by blood nucleases and binding

proteins. Interestingly, defects in intracellular and extracellular nucleases can predispose to autoimmunity, presumably by increasing the amount of immunoactive DNA in the system [15-17].

2.2 The assay of extracellular DNA

In view of the structure of DNA and its existence in the form of nucleosomes, assay of extracellular DNA can be accomplished by biochemical or molecular assays of DNA itself or assay of nucleosomes in blood; these assays use serum or plasma in which cellular elements, including platelets, are removed by differential centrifugation to produce a cell-free (cf) preparation. Assays of cfDNA can involve binding of fluorimetric dyes such as PicoGreen or polymerase chain reaction (PCR) amplification of specific sequences from purified cfDNA. The fluorimetric assays are simple and inexpensive although possibly limited by the effect of protein binding on fluorescence yield of any dye. PCR amplification of specific sequences is precise but results could be potentially confounded by selective loss of a particular gene during apoptosis, for example. Immunoassay of nucleosomes involves “sandwich” assays whose performance characteristics depend on the specificity and properties of the capture and developing antibodies [18-22].

2.3 Generation of the Blood Nucleome

The blood is a rich source of extracellular nucleic acids that comprise what can be termed the blood nucleome by analogy with the proteome and transcriptome. DNA is a major component of the nucleome and, while RNA is also present, RNA is more labile and subject to nuclease digestion than DNA. In addition to DNA and RNA, the blood nucleome contains nuclear proteins including histones and HMGB1. As now recognized, cytokines such as IL-1 α and IL-33 also originate in the cell nucleus and can be categorized under this rubric [23,24]. Even in normal people, blood contains significant concentrations of DNA, likely resulting from the large number of cells that die each day.

Among diseases characterized by immune responses to DNA, systemic lupus erythematosus (SLE) was one of the first settings in which the expression of blood DNA was explored. SLE is a prototypic autoimmune disease characterized by antibodies to components of the cell nucleus (antinuclear antibodies or ANAs), with anti-DNA antibodies important markers for diagnosis, prognosis and disease activity. Levels of anti-DNA can rise and fall with disease activity, with elevations of anti-DNA levels closely associated with nephritis in many patients. Nephritis results from the deposition of immune complexes comprised of DNA as well as any associated proteins; localization of these complexes to the kidney may result from their predilection for charged sites in the glomerular basement membrane [25,26].

While DNA can be elevated in the blood of lupus patients, subsequent studies indicated that elevations of DNA are not specific for this setting even though anti-DNA antibody production may be essentially exclusive to lupus [27]. Thus, using a variety of analytic techniques, studies demonstrated elevated levels of DNA in the blood many different clinical settings (Table 1). Such conditions range from pregnancy to malignancy although all appear associated with inflammation or aberrant or accentuated levels of cell death. DNA thus represents an analyte that can be measured as a biomarker of cell death, providing a general assessment of death processes [28].

As now recognized, cell death can follow various patterns that differ in immunological consequences [2]. For this discussion, however, death can be divided into three main patterns with respect to their potential contribution to DNA in the blood nucleome: apoptosis, necrosis and NETosis. Apoptosis is a regulated form of cell death mediated by

enzymatic cascades triggered intrinsically or extrinsically. Necrosis is an accidental form of cell death induced by chemical or physical injury; depending on the trigger, however, necrosis can be regulated and proceed under the control of enzymatic processes. Finally, NETosis appears limited to certain hematopoietic cell types (e.g., neutrophils) and is a rapid, almost explosive process to disgorge DNA, with attached cytoplasmic and granule proteins, as an anti-bacterial defense [7-9].

Reflecting the differences in death mechanisms, DNA that emerges from dead and dying cells can vary markedly in size. In apoptosis, nucleases cleave and degrade DNA and produce low molecular weight species including ladders. In necrosis, especially the more rapid forms that follow extreme trauma, DNA emerges from cells otherwise intact and has high molecular weight. Similarly, during NETosis, DNA has high molecular weight to allow generation of a lattice or mesh. The differences in the size of the DNA in the blood can be determined by gel electrophoresis or PCR amplification of the same gene using primers separated by different lengths of DNA [18,29]. Depending on the separation between the primers, the integrity of DNA in the blood can be assessed [29].

To elucidate the mechanisms for the generation of extracellular DNA, our laboratory has characterized *in vitro* and *in vivo* models. Studies *in vitro* have involved long-term cell lines induced to undergo either apoptosis or necrosis, measuring DNA by the dye PicoGreen. As shown in these experiments, with apoptosis, cell lines, such as the Jurkat T cell leukemia, release DNA in a time-dependent process, with extracellular DNA emerging during late apoptosis or secondary necrosis. This DNA shows laddering. With necrosis *in vitro*, the situation is more variable, with the amounts and time course of DNA release dependent on the inducing stimulus. With death induced by freeze-thaw (a commonly used technique for studying the immunological activities of necrotic cells), DNA release from cells is almost immediate but subject to nuclease degradation. In contrast, DNA release from cells undergoing necrosis with heat or H2O2 treatment is much lower and slower; DNA release from cells treated with ethanol is minimal [30-32].

Modeling cell death *in vivo* is more complicated since agents intended to kill a specific cell type (e.g., hepatocyte) can have more widespread cellular toxicity and injure multiple cell types [33]. As a simple model, we therefore administered cells killed *ex vivo* (either apoptosis or necrosis) into normal mice by the intraperitoneal (IP) route and measured DNA in the blood [34]. For these experiments, we used Jurkat cells since they are derived from a male and therefore have a Y-chromosome to allow unequivocal identification of the origin of blood DNA by PCR amplification when cells are administered to a female recipient. Together, these studies showed that the administration of either apoptotic or necrotic Jurkat cells leads to high blood levels of DNA that occur within 5-6 hours of cell administration and disappear by 24 hours. This DNA bears sequences of the Y chromosome and shows laddering irrespective of whether the cells died by apoptosis or necrosis *in vitro*. These findings suggest that laddering can reflect events after cell death, such as digestion by nucleases from serum or engulfing phagocytes [34].

Subsequent studies showed that macrophages are required for a blood DNA response. Thus, in mice treated with clodronate liposomes to deplete macrophages, DNA did not appear in the blood following administration of either apoptotic or necrotic Jurkat cells [34]. These results are surprising since they suggest that the presence of a large number of dead and dying cells is not sufficient to generate a blood DNA response; rather, this response appear to require an interaction of dead and dying cells with phagocytes. In this scenario, the macrophage may engulf and digest the dead and dying cells and discharge the DNA, cleaved into lower molecular weight fragments, as a waste product. Alternatively, the burst of DNA in the blood could represent a failed attempt by macrophages to clear the dead and dying

cells; with the capacity of phagocytosis exceeded, the macrophage could die and release both its DNA and the DNA of engulfed Jurkat cells. Interestingly, in this model, male animals show much lower levels of DNA than do female mice. [35].

A rise in blood DNA can also follow *in vivo* interventions to induce inflammation or cell death in various organs *in situ*. Thus, treatment of mice with hepatotoxic agents such as acetaminophen, carbon tetrachloride or anti-Fas all cause a rapid rise in DNA that is similar in amounts and kinetics to that resulting from the administration of cells killed *ex vivo* [36]. Since acetaminophen, carbon tetrachloride and anti-Fas all rapidly kill large number of hepatocytes (and possibly other cell populations), a resemblance to the administration of dead cells is not unexpected; nevertheless, the difference in the location of cell death could impact on this response. With the hepatotoxins, DNA in the blood shows a low molecular weight consistent with nucleosomal dimensions, suggesting that laddering can occur with either apoptosis or necrosis [36].

Another experimental model that leads to the release of DNA into the blood involves endotoxin administration, especially at doses that cause shock [33,37,38]. Endotoxin has multiple actions that can encompass inflammation as well as cell death, with at least some death resulting from ischemia and circulatory collapse. With endotoxin as well as other models of septic shock (e.g., cecal ligation and perforation), formation of NETs may occur, with this event leading to at least some of the DNA in the blood. While the source of DNA that emerges in the blood in sepsis is not clear (with possible contributions of parenchymal and immune cell death as well as NETs formation), sepsis can associated with high levels of blood DNA. In this regard, sepsis models are also characterized by high levels of other nuclear components such as histones and HMGB1 in the blood [39,40].

Studies on the release on the extracellular release of DNA either *in vivo* or *in vitro* indicate that at least some of the DNA that can be released from dead and dying cells exists in the form of microparticles [41-43]. Microparticles are small membrane-bound vesicles that emanate from the cell membrane of both activated and dying cells by a blebbing processes [44,45]. These particles are approximately 0.2 to 1.0 um in diameter and contain a sampling of membrane, cytoplasmic and nuclear components. Importantly, MPs appear to be an important source of extracellular nuclear molecules since, during apoptosis, nuclear molecule migrate into blebs for eventual release into the extracellular space. While the basis of blebbing and particle release is not known, MP generation resemble the formation of apoptotic bodies and provide a smaller and more “digestible” structure to facilitate removal of dead cell debris.

Reflecting an origin of MPs from apoptotic cells, DNA in particles shows cleavage and laddering [43]. Importantly, the nuclear material in particles is accessible for binding to antinuclear antibodies, including monoclonal anti-DNA and anti-histones as well as serum autoantibodies [46]. The antigenicity of particles results from the display of nucleosomal components on the surface or the porosity of particle structure that allows entry of antibodies. As such, microparticles can form immune complexes. As shown by flow cytometry, blood of patients with lupus shows increased numbers of microparticles with bound IgG, although the role of these particles in pathogenesis is speculative at this time [46].

2.4 Immune Activation by DNA

Since the original discovery of the role of DNA as a central autoantigen in SLE, the conceptualization of DNA's immune properties has undergone a remarkable transformation. As shown in studies in both patients and murine models of lupus, the autoantibody response to DNA shows features of an antigen-driven response as evidenced by the clonality of

antibodies and patterns of somatic mutation. These studies further suggest that DNA itself is the selecting antigen since some of the mutations (i.e., CDR3 arginines) can augment DNA interaction [47,48]. Nevertheless, in experimental immunization models in animals, DNA (double stranded mammalian DNA in the B conformation) shows at best very limited immunogenicity even if presented in association with a protein carrier in complete Freund's adjuvant. Together, these studies led to the idea that DNA is essentially inert outside the setting of lupus where B and T cell disturbances may allow recognition of a molecule that is ordinarily invisible to the immune system [1].

In the last two decades, studies in a number of realms have led to a vastly different picture of DNA's immune properties and the recognition that base sequence has an enormous impact on activity. As shown originally with mycobacterial and bacterial DNA as well as synthetic oligonucleotides, DNA can have powerful immune properties as shown in *in vitro* culture systems of macrophages, dendritic cells and B cells from humans as well animals. Furthermore, administration of DNA to animals can replicate these activities, establishing in *in vivo* relevance of the *in vitro* findings. As shown in seminal studies, stimulation by foreign DNA results from sequences called CpG motifs in which an unmethylated cytosine residue is followed by guanosine. CpG motifs occur much more commonly in prokaryotic than in eukaryotic DNA because of differences in patterns of base methylation as well as the underrepresentation of CpG dinucleotides in mammalian DNA, a phenomenon called CpG suppression [49].

CpG motifs represent a distinct molecular signature that allows foreign DNA to act as a pathogen associated molecular pattern or PAMP. DNA with such sequences is often termed CpG DNA although its origin (natural vs. synthetic) can vary. While bacterial DNA can serve as a PAMP, its role in inducing responses during infection is not known. In this regard, the blood has a high content of DNases which theoretically could digest foreign DNA and reduce its potential role in settings such infection and sepsis. Interestingly, bacteria also carry nucleases which may be part of their arsenal of measures against host defense by digesting NETs [50].

In contrast to the immune activity of CpG DNA, mammalian DNA, even though it may contain some CpG motifs, is inactive in *in vitro* models. Furthermore, mammalian DNA can inhibit the response to CpG DNA, a property that may reflect certain G-rich sequences that occur in telomeric regions of the chromosome [51,52]. The inhibitory activity of mammalian DNA may account for the failure of immunization models to generate anti-DNA production. Importantly, bacterial DNA is an effective immunogen in animal models, eliciting antibodies that are specific for bacterial DNA sequences in normal mice and cross-reactive anti-DNA autoantibodies in pre-autoimmune NZB/NZW mice [53].

The demonstration of CpG DNA's activity as a PAMP led rapidly to the discovery to TLR9 as a key DNA sensor in B cells, dendritic cells and macrophages and the critical role of MyD88 as a downstream signaling element [54,55]. Importantly, as subsequent studies showed, TLR9, along with TLR3 and TLR 7/8 (sensors for double stranded and single stranded RNA respectively) function in an intracellular location and thus differ from TLRs on the cell surface [56,57]. An intracellular location is consistent with the role of these receptors in the host response to DNA and RNA from intracellular infection; an intracellular location could also prevent inadvertent stimulation by DNA circulating in the blood nucleome. Nevertheless, extracellular nucleic acids can access these TLRs following uptake into an endosomal compartment in a pH sensitive process that can be interdicted by antimalarial agents. The effect of anti-malarials is notable in view of their use in the treatment of lupus and other rheumatic disease characterized by anti-nuclear antibodies [58,59].

Studies to characterize immune stimulation by DNA have frequently used as model compounds synthetic oligonucleotides bearing CpG motifs, often with a phosphorothioate backbone. Phosphorothioates have a sulfur substitution for a non-bridging oxygen in the backbone. This substitution increases resistance to nuclease digestion and enhances both protein binding and cellular uptake. While these studies have been valuable in developing novel agents with immunostimulatory (and immunosuppressive) properties and defining structures that can stimulate different cell populations, a small oligonucleotide can show different patterns of cellular entry, intracellular trafficking and protein interactions in comparison with an extended polynucleotide with a phosphodiester backbone. Nevertheless, many aspects of stimulation appear the same with oligonucleotides and polynucleotides although the active sequences of phosphodiester and phosphorothioate compounds may differ [60-62].

Another format to assess immune activation by DNA involves the culture of cells with DNA mixed with an agent to promote DNA uptake into cells. Such agents, which are commonly used for transfection of cells for gene expression studies, are comprised of lipid mixtures and can form nanostructures to allow delivery of DNA into cells by endocytosis [63,64]. Such agents allow DNA access into other compartments than would not occur with incubation of cells with free DNA or oligonucleotides. The resulting stimulation involves non-TLR sensors such as DAI (DNA activator of IFN-regulatory factors), also called zBP1 [65]. Importantly, stimulation of the cells by transfected DNA does not require CpG motifs and appears to occur with essentially any DNA; even mammalian DNA, which would be inactive or inhibitory when cultured in a free form, can stimulate response when presented to cells in this manner.

By analogy with transfection agents, naturally occurring proteins can also affect immune stimulation by DNA. Thus, DNA autoantibodies can form immune complexes with DNA, either natural or synthetic, and dramatically enhance stimulation of plasmacytoid dendritic cells in particular when compared to free DNA [66-68]. This stimulation may be important in the pathogenesis of lupus since it leads to production of type I interferon whose key role in lupus has been inferred from the “interferon signature” of peripheral blood cells of patients; type I interferon has actions that can impact on the both immune cells and endothelial cells and could drive vascular complications as well as immune abnormalities [69]. LL37, a cathelicidin, is another protein that can bind DNA and enhance its immunostimulatory activity, presumably by facilitating cellular uptake; this activity may be relevant to various diseases such as psoriasis and atherosclerosis where LL37 expression occurs in involved tissues [70,71]. In the case of immune complexes of DNA and anti-DNA, immune stimulation involves both TLR9 and non-TLR nucleic acid sensors, with antibody presumably providing access to a broader array of sensors than would free DNA [66-68].

2.5 The Role of HMGB1 in Immune Stimulation by DNA

In contrast to results with model complexes created *in vitro* by mixing pure DNA with antibody, studies with DNA-containing immune complexes from patients demonstrate involvement of some unexpected players. Thus, stimulation of PDCs by immune complexes obtained from patients with lupus involves RAGE, the receptor for advanced glycation end-products. The involvement of RAGE reflects the presence of HMGB1 which can bind to DNA and appears in the circulation under similar conditions as does DNA. As now recognized, HMGB1, following release from cells, can mediate the immune response to cell death as well as act downstream of PAMPs such as LPS [72,73]. A highly conserved molecule, HMGB1 is a non-histone nuclear proteins that is comprised of two DNA binding domains called the A box and B box as well as C terminal tail. While a major nuclear component, HMGB1 is not ordinarily tightly bound to DNA and has considerable mobility

inside the cell, translocating from nucleus to cytoplasm depending on the state of the cell [40].

As shown in illuminating experiments in the past few years, while HMGB1 is considered as classic alarmin or DAMP (damage associated molecular pattern), its immunological activity and expression in inflammation are nevertheless variable and complex. HMGB1 can translocate to the outside of cells during activation and cell death although its immunological activity depends on its redox state and the status of three cysteine residues [74-76]. In its reduced form, HMGB1 can trigger responses via TLR4 to which it binds while, with oxidation, the immune activity of HMGB1 is lost. Among its other activities, HMGB1 can bind to LPS to induce responses. HMGB1 also can bind to cytokines such as IL-1 β and trigger cells via a cytokine receptors [77,78]. The panoply of these interactions reflects the sequence of HMGB1 that allows creation of a potentially wide array of immunostimulatory structures that in fact may include complexes with DNA [79].

While DNA and HMGB1 are both intrinsic nuclear components, the impact of HMGB1 on the immune response to DNA may relate to the attachment of HMGB1 to chromatin and changes that occur during cell death. Original studies indicated marked differences in the extent of HMGB1 release during *in vitro* necrosis and apoptosis of cell lines. Using Western blotting to assay HMGB1 in culture media, these studies demonstrated extensive release of HMGB1 during necrosis; this release most likely occurs passively since HMGB1 is not tightly adherent to chromatin and can diffuse away when cell permeability barriers breakdown. In contrast, in these studies, HMGB1 remained immobilized in the cell nucleus during apoptosis, likely because of post-translational modifications that increase chromatin binding [74,75]. Such a dichotomy would be consistent with divergent activity of necrotic and apoptotic cells, with necrotic cells pro-inflammatory and apoptotic cells anti-inflammatory. While subsequent studies showed that HMGB1 can exit apoptotic cells during secondary necrosis, the oxidation state would prevent any immune stimulation [80,81].

Studies on lupus mice have produced an intriguing picture on how HMGB1 could influence the response to DNA during autoimmunity. Thus, Urbanaviciute, et al [82] demonstrated that complexes of HMGB1 and nucleosomes could stimulate dendritic cells and macrophages to produce cytokines such as IL-1 β , IL-6 and TNF- α as well as upregulation of co-stimulatory molecules under conditions in which neither component alone is active. Furthermore, HMGB1-containing nucleosomes prepared from apoptotic cells could induce the production of anti-DNA and anti-histones in normal mice by immunization; nucleosomes from living cells failed to induce such responses. Importantly, the induction of anti-nuclear antibodies by this form of immunization depends on TLR2, likely reflecting the key role of HMGB1 in creating adjuvant activity.

Together, these studies suggest that immune stimulation by extracellular DNA in lupus and possibly other clinical settings is mutable and can entail large structures in which individual components may themselves have little or no activity [79]. These structures would consist of DNA, histones, non-histone nuclear proteins and potentially cytoplasmic proteins, with composition affected by the cell of origin and death mechanism (e.g., NETosis) [83]. These complexes could be pre-formed in the cell although possibly modified during apoptosis or NETosis by post-translational modification; alternatively, the complexes could form from components that would interact in the extracellular space even if they exited from cells separately. Figure 1 depicts the extracellular release of DNA during apoptosis and subsequent formation of immune complexes.

Formally, mammalian DNA itself may not be a DAMP, since many studies have indicated that it lacks activity alone and indeed may inhibit responses to other stimuli including foreign DNA. This categorization may not be informative, however, since, as noted, DNA may not ordinarily exist in a free state. Furthermore, DNA has biochemical properties to allow creation of a DAMP by binding with another molecule especially in setting of cell death where a host of DAMPs is present. A term for these molecules has not yet been developed although partial (or incomplete) DAMP, proto-DAMP or precursor DAMP can all be considered. Table 2 summarizes the immune properties of DNA.

3. Implications for Treatment

In the context of SLE, DNA (in whatever form it takes *in vivo*) can drive both non-specific immune responses (e.g., interferon production) as well as specific immune responses (i.e., anti-DNA autoantibody production). While these responses can be analyzed separately, they may in fact be closely linked since the non-specific immunostimulatory activities of DNA may promote specific autoantibody responses. Indeed, the non-specific immune properties of DNA may allow DNA to serve as an adjuvant in which the same molecule displays both adjuvant and antigenic properties although for DNA, the associated molecules (e.g., HMGB1) is necessary for the adjuvant activity. Furthermore, since HMGB1 can bind cytokines such as IL-1 β and IFN- γ , the immunogenicity of DNA could be further enhanced by its association with HMGB1 and its own companion adjuvant partners [77,78, 83].

Considering DNA to operate in the context of a complex opens up a wide variety of possibilities for new targets of therapy. Thus, therapies directed at the DNA itself include inhibitors of TLR9 as well as DNA-binding polymers that can scavenge DNA and prevent its interaction with either proteins as well as entry to cells [84]. Therapies directed at HMGB1 include antibodies; B box construct that can inhibit immune activity of HMGB1; antibodies to RAGE; and strategies to block the TLRs with which it can interact [85]. For stimulation by an HMGB1-nucleosome complex, TLRs which could be inhibited include TLR2 and TLR 4 as well as TLR9. At present, the role of other TLRs in these responses is not clear. Therapies directed at non-TLR receptors, including the inflammasome, are also possible although specific agents for this purpose have not yet been explored.

One approach to therapy that has not yet received attention concerns antibodies to the DNA and histone components of complexes. Studies have indicated that antibodies to histones can block immune activation and thrombosis in various murine models including liver injury although the range of antibodies to different nucleosomal antigens with these properties has not been explored [86]. Studies have suggested that, among anti-DNA monoclonal antibodies, some can cause renal disease by transfer into normal mice and some cannot [87]. Furthermore, some lupus patients have high levels of anti-DNA antibodies without nephritis; similarly, some mice strains of lupus mice have high anti-DNA without nephritis. Together, these studies have suggested that the ability of an anti-DNA antibody to cause nephritis reflects a specific pattern of antigen interaction either to allow immune complex formation, complement fixation or renal localization. Studies have not addressed whether non-pathogenic or non-nephritogenic anti-DNA antibodies can actually protect against disease as in the case of anti-HMGB1 antibodies or certain anti-histone antibodies. Future studies will hopefully address the intriguing possibility that autoantibodies can be mechanism to attenuate immune activation of DNA in a way that can be exploited for therapy.

4. Conclusion

In conclusion, in its role in the nucleus, DNA has an extraordinary capacity for intermolecular interaction reflecting its charge and the myriad structural motifs provided by

backbone conformation, base sequence as well as epigenetic modification. DNA does not lose this capacity when it enters the extracellular space and, as in the case of HMGB1, DNA loves company [88]. Future studies will define this company and its impact of the properties of DNA in normal and aberrant immunity.

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REFERENCES

- [1]. Pisetsky DS. Immune activation by bacterial DNA: a new genetic code. *Immunity*. 1996; 5:303–310. [PubMed: 8885863]
- [2]. Griffith TS, Ferguson TA. Cell death in the maintenance and abrogation of tolerance: the five Ws of dying cells. *Immunity*. 2011; 35:456–466. [PubMed: 22035838]
- [3]. Hansen JD, Vojtech LN, Laing KJ. Sensing disease and danger: a survey of vertebrate PRRs and their origins. *Dev Comp Immunol*. 2011; 35:886–897. [PubMed: 21241729]
- [4]. Kawasaki T, Kawai T, Akira S. Recognition of nucleic acids by pattern-recognition receptors and its relevance in autoimmunity. *Immunol Rev*. 2011; 243:61–73. [PubMed: 21884167]
- [5]. Ranjin P, Bowzard JB, Schwerzmann JW, Jeisy-Scott V, Fujita T, Sambhara S. Cytoplasmic nucleic acid sensors in antiviral immunity. *Trends Mol Med*. 2009; 15:359–368. [PubMed: 19665430]
- [6]. Theofilopoulos AN, Kono DH, Beutler B, Baccala R. Intracellular nucleic acid sensors and autoimmunity. *J Interferon Cytokine Res*. 2011; 31:867–886. [PubMed: 22029446]
- [7]. Brinkmann V, Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. *Nature Rev Microbiol*. 2007; 5:577–582. [PubMed: 17632569]
- [8]. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol*. 2007; 176:231–241. [PubMed: 17210947]
- [9]. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol*. 2010; 191:677–691. [PubMed: 20974816]
- [10]. Khorasanizadeh S. The nucleosome: from genomic organization to genomic regulation. *Cell*. 2004; 116:259–272. [PubMed: 14744436]
- [11]. Brown DG, Sun X-M, Cohen GM. Dexamethasone-induced apoptosis involves cleavage of DNA to large fragments prior to internucleosomal fragmentation. *J Biol Chem*. 1993; 268:3037–3039. [PubMed: 8428979]
- [12]. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature*. 1998; 391:43–50. [PubMed: 9422506]
- [13]. Nagata S. Apoptotic DNA fragmentation. *Exp Cell Res*. 2000; 256:12–18. [PubMed: 10739646]
- [14]. Samejima K, Earnshaw WC. Trashing the genome: the role of nucleases during apoptosis. *Nat Rev Mol Cell Biol*. 2005; 6:677–688. [PubMed: 16103871]
- [15]. Napirei M, Karsunky H, Zevnik B, Stephean H, Mannherz HG, Möröy T. Features of systemic lupus erythematosus in Dnase1-deficient mice. *Nat Genet*. 2000; 25:177–181. [PubMed: 10835632]
- [16]. Zykova SN, Tveita AA, Rekvig OP. Renal Dnase1 enzyme activity and protein expression is selectively shut down in murine and human membranoproliferative lupus nephritis. *PLoS One*. 2010; 10:e12096. [PubMed: 20856893]
- [17]. Jog NR, Frisoni L, Monestier M, Hernandez S, Craft J, Prak ET, Caricchio R. Caspase-activated DNase is required for maintenance of tolerance to lupus nuclear autoantigens. *Arthritis Rheum*. 2012; 64:1247–1256. [PubMed: 22127758]

- [18]. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch R-D, Knippers R. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. *Cancer Res.* 2001; 61:1659–1665. [PubMed: 11245480]
- [19]. Björkman L, Reich CF 3rd, Pisetsky DS. The use of fluorometric assays to assess the immune response to DNA in murine systemic lupus erythematosus. *Scand J Immunol.* 2003; 57:525–533. [PubMed: 12791090]
- [20]. Holdenrieder S, Stieber P, Chan LYS, Geiger S, Kremer A, Nagel D, Dennis Lo YM. Cell-free DNA in serum and plasma: comparison of ELISA and quantitative PCR. *Clin Chem.* 2005:1544–1546. [PubMed: 16040855]
- [21]. Jung K, Fleischhacker M, Rabien A. Cell-free DNA in the blood as a solid tumor-biomarker-a critical appraisal of the literature. *Clinica Chimica Acta.* 2010; 411:1611–1624.
- [22]. Holdenrieder S, Stieber P. Clinical use of circulating nucleosomes. *Crit Rev Clin Lab Sci.* 2009; 46:1–24. [PubMed: 19107649]
- [23]. Moussion C, Ortega N, Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel ‘alarmin’? *PLoS One.* 2008; 6:e3331. [PubMed: 18836528]
- [24]. Cohen I, Rider P, Carmi Y, Braiman A, Dotan S, White MR, Voronov E, Martin MU, Dinarello CA, Apte RN. Differential release of chromatin-bound IL-1 α discriminates between necrotic and apoptotic cell death by the ability to induce sterile inflammation. *Proc Natl Acad Sci U S A.* 2010; 107:2574–2579. [PubMed: 20133797]
- [25]. Hahn BH. Antibodies to DNA. *N Engl J Med.* 1998; 338:1359–1368. [PubMed: 9571257]
- [26]. Jang YJ, Stollar BD. Anti-DNA antibodies: aspects of structure and pathogenicity. *Cell Mol Life Sci.* 2003; 60:309–320. [PubMed: 12678496]
- [27]. Lui YYN, Lo YMD. Circulating DNA in plasma and serum: biology, preanalytical issues and diagnostic applications. *Clin Chem Lab Med.* 2002; 40:962–968. [PubMed: 12476933]
- [28]. Lam NYL, Rainer TH, Chan LYS, Joynt GJ, Lo YMD. Time course of early and late changes in plasma DNA in trauma patients. *Clin Chem.* 2003; 49:1286–1291. [PubMed: 12881444]
- [29]. Wang BG, Huang H-Y, Chen Y-C, Bristow RE, Kassaei K, Cheng C-C, Roden R, Sokill LJ, Chan DW, Shih I-M. Increased plasma DNA integrity in cancer patients. *Cancer Res.* 2003; 63:3966–3968. [PubMed: 12873992]
- [30]. Choi JJ, Reich CF 3rd, Pisetsky DS. Release of DNA from dead and dying lymphocyte and monocyte cell lines in vitro. *Scand J Immunol.* 2004; 60:159–166. [PubMed: 15238085]
- [31]. Choi JJ, Reich CF 3rd, Pisetsky DS. The role of macrophages in the in vitro generation of extracellular DNA from apoptotic and necrotic cells. *Immunology.* 2005; 115:55–62. [PubMed: 15819697]
- [32]. Beyer C, Stearns NA, Giessel A, Distler JH, Schett G, Pisetsky D. The extracellular release of DNA and HMGB1 from Jurkat T cells during in vitro necrotic cell death. *Innate Immun.* Feb 16.2012 Epub ahead of print.
- [33]. Jiang N, Reich CF 3rd, Monestier M, Pisetsky DS. The expression of plasma nucleosomes in mice undergoing in vivo apoptosis. *Clin Immunol.* 2003; 106:139–147. [PubMed: 12672404]
- [34]. Jiang N, Reich CF 3rd, Pisetsky DS. Role of macrophages in the generation of circulating blood nucleosomes from dead and dying cells. *Blood.* 2003; 102:2243–2250. [PubMed: 12775567]
- [35]. Pisetsky DS, Jiang N. The generation of extracellular DNA in SLE: the role of death and sex. *Scand J Immunol.* 2006; 64:200–204. [PubMed: 16918687]
- [36]. Tran TT, Groben P, Pisetsky DS. The release of DNA into the plasma of mice following hepatic cell death by apoptosis and necrosis. *Biomarkers.* 2008; 13:184–200. [PubMed: 18270870]
- [37]. Fourmé GJ, Lambert PH, Meischer PA. Release of DNA in circulating blood and induction of anti-DNA antibodies after injection of bacterial lipopolysaccharides. *J Exp Med.* 1974; 140:1189–1206. [PubMed: 4607609]
- [38]. Licht R, vanBruggen MC, Oppers-Walgreen B, Rijke TP, Berden JH. Plasma levels of nucleosomes and nucleosome-autoantibody complexes in murine lupus: effects of disease progression and lipopolysaccharide administration. *Arthritis Rheum.* 2001; 44:1320–1330. [PubMed: 11407691]

- [39]. Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semararo F, Taylor FB, Esmon NL, Lupu F, Esmon CT. Extracellular histones are major mediators of death in sepsis. *Nature Med.* 2009; 15:1318–1322. [PubMed: 19855397]
- [40]. Harris HE, Andersson U, Pisetsky DS. HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. *Nat Rev Rheumatol.* 2012 doi: 10.1038/nrrheum.2011.222. [Epub ahead of print].
- [41]. Casciola-Rosen LA, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J Exp Med.* 1994; 179:1317–1330. [PubMed: 7511686]
- [42]. Schiller M, Bekeredian-Ding I, Heyder P, Ho AD, Lorenz HM. Autoantigens are translocated into small apoptotic bodies during early stages of apoptosis. *Cell Death Differ.* 2008; 15:183–191. [PubMed: 17932498]
- [43]. Reich CF 3rd, Pisetsky DS. The content of DNA and RNA in microparticles released by Jurkat and HL-60 cells undergoing in vitro apoptosis. *Exp Cell Res.* 2009; 315:760–768. [PubMed: 19146850]
- [44]. Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ Res.* 2010; 107:1047–1057. [PubMed: 21030722]
- [45]. Pisetsky DS, Ullal AJ, Gauley J, Ning TC. Microparticles as mediators and biomarkers of rheumatic disease. *Rheumatology.* Mar 7.2012 E pub ahead of print.
- [46]. Ullal AJ, Reich CF 3rd, Clowse M, Criscione-Schreiber LG, Tochacek M, Monestier M, Pisetsky DS. Microparticles as antigenic targets of antibodies to DNA and nucleosomes in systemic lupus erythematosus. *J Autoimmun.* 2011; 36:173–180. [PubMed: 21376534]
- [47]. Radic MZ, Weigert M. Genetic and structural evidence for antigen selection of anti-DNA antibodies. *Annu Rev Immunol.* 1994; 12:487–520. [PubMed: 8011289]
- [48]. Guo W, Smith D, Aviszus K, Detanico T, Heiser RA, Wysocki LJ. Somatic hypermutation as a generator of antinuclear antibodies in a murine model of systemic autoimmunity. *J Exp Med.* 2010; 207:2225–2237. [PubMed: 20805563]
- [49]. Krieg AM. CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol.* 2002; 20:709–760. [PubMed: 11861616]
- [50]. Berends ET, Horswill AR, haste NM, Monestier M, Nizet V, von Kückritz-Blickwede M. Nuclease expression by staphylococcus aureus facilitates escape from neutrophil extracellular traps. *J Innate Immun.* 2010; 2:576–586. [PubMed: 20829609]
- [51]. Pisetsky DS, Reich CF 3rd. Inhibition of murine macrophage IL-12 production by natural and synthetic DNA. *Clin Immunol.* 2000; 96:198–204. [PubMed: 10964537]
- [52]. Gursel I, Gursel M, Yamada H, Ishii KJ, Takeshita F, Klinman DM. Repetitive elements in mammalian telomeres suppress bacterial DNA-induced immune activation. *J Immunol.* 2003; 171:1393–1400. [PubMed: 12874230]
- [53]. Gilkeson GS, Pippen AM, Pisetsky DS. Induction of cross-reactive anti-dsDNA antibodies in preautoimmune NZB/NZW mice by immunization with bacterial DNA. *J Clin Invest.* 1995; 95:1398–1402. [PubMed: 7883986]
- [54]. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S. A toll-like receptor recognizes bacterial DNA. *Nature.* 2000; 408:740–745. [PubMed: 11130078]
- [55]. Bauer S, Kirschning CJ, Häcker H, Redecke V, Hausmann S, Akira S, Wagner H, Lipford GB. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci U S A.* 2001; 98:9237–9242. [PubMed: 11470918]
- [56]. Rutz M, Metzger J, Gellert T, Lippa P, Lipford GB, Wagner H, Bauer S. Toll-like receptor 9 binds single-stranded CpG-DNA in a sequence- and pH-dependent manner. *Eur J Immunol.* 2004; 34:2541–2550. [PubMed: 15307186]
- [57]. Akira S, Takeda K. Toll-like receptor signaling. *Nat Rev Immunol.* 2004; 4:499–511. [PubMed: 15229469]
- [58]. Lee SJ, Silverman E, Bargman JM. The role of antimalarial agents in the treatment of SLE and lupus nephritis. *Nat Rev Rev Nephrol.* 2011; 7:718–729.

- [59]. Ben-Zvi I, Kivity S, Langevitz P, Shoenfeld Y. Hydroxychloroquine: from malaria to autoimmunity. *Clin Rev Allerg Immunol*. 2012; 42:145–153.
- [60]. Pisetsky DS, Reich CF 3rd. Influence of backbone chemistry on immune activation by synthetic oligonucleotides. *Biochem Pharmacol*. 1999; 58:1981–1988. [PubMed: 10591154]
- [61]. Klinman D, Shirota H, Tross D, Sato T, Klaschik S. Synthetic oligonucleotides as modulators of inflammation. *J Leukoc Biol*. 2008; 84:958–964. [PubMed: 18430787]
- [62]. Krieg AM. CpG still rocks! Update on an accidental drug. *Nucleic Acid Ther*. Feb 21.2012 Epub ahead of print.
- [63]. Jiang W, Reich CF 3rd, Pisetsky DS. Mechanisms of activation of the RAW 264.7 macrophage cell line by transfected mammalian DNA. *Cell Immunol*. 2004; 229:31–40. [PubMed: 15331326]
- [64]. Jiang W, Pisetsky DS. The induction of HMGB1 release from RAW 264.7 cells by transfected DNA. *Mol Immunol*. 2008; 45:2038–2044. [PubMed: 18031817]
- [65]. Choubey D. DNA-responsive inflammasomes and their regulators in autoimmunity. *Clin Immunol*. 2012; 142:223–231. [PubMed: 22245264]
- [66]. Vallin H, Perers A, Alm GV, Rönnblom L. Anti-double-stranded DNA antibodies and immunostimulatory plasmid DNA in combination mimic the endogenous IFN- α inducer in systemic lupus erythematosus. *J Immunol*. 1999; 163:6306–6313. [PubMed: 10570325]
- [67]. Boulé MW, Broughton C, Mackay F, Akira S, Marshak-Rothstein A, Rifkin IR. Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes. *J Exp Med*. 2004; 199:1631–1640. [PubMed: 15197227]
- [68]. Tian J, Avalos AM, Mao S-Y, Chen B, Senthil K, Wu H, Parroche P, Drabic S, Golenbock D, Sirois C, Hua J, An LL, Audoly L, LaRosa G, Bierhaus A, Naworth P, Marshak-Rothstein A, Crow MK, Fitzgerald KA, Latz E, Kiener PA, Coyle AJ. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nature Immunol*. 2007; 8:487–497. [PubMed: 17417641]
- [69]. Rönnblom L, Eloranta M-L, Alm GV. The type I interferon system in systemic lupus erythematosus. *Arthritis Rheum*. 2006; 54:408–420. [PubMed: 16447217]
- [70]. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zal T, Mellman I, Schröder JM, Liu YJ, Gilliet M. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature*. 2007; 449:564–569. [PubMed: 17873860]
- [71]. Döring Y, Manthey HD, Dreschler M, Lievens D, Megens RT, Soehnlen O, Busch M, manca M, Koenen RR, Pelisek J, Daemen MJ, Lutgens E, Zenke M, Binder CJ, Weber C, Zernecke Z. Auto-antigenic protein-DNA complexes stimulate plasmacytoid dendritic cells to promote atherosclerosis. *Circulation*. 2012; 125:1673–1683. [PubMed: 22388324]
- [72]. Wang H, Bloom O, Zhang M, Vishnubakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ. HMG-1 as a late mediator of endotoxin lethality in mice. *Science*. 1999; 285:248–251. [PubMed: 10398600]
- [73]. Andersson U, Harris HE. The role of HMGB1 in the pathogenesis of rheumatic disease. *Biochim Biophys Acta*. 2010; 1799:141–148. [PubMed: 20123076]
- [74]. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature*. 2002; 418:191–195. [PubMed: 12110890]
- [75]. Rovere-Querini P, Capobianco A, Scaffidi P, Valentini B, Catalanotti F, Giazson M, Dumitriu IE, Müller S, Iannacone M, Traversari C, Bianchi ME, Manfredi AA. HMGB1 is an endogenous immune adjuvant released by necrotic cells. *EMBO Rep*. 2004; 5:825–830. [PubMed: 15272298]
- [76]. Yang H, Lundbäck P, Ottosson L, Erlandsson-Harris H, Venereau E, Bianchi ME, Al-Abed Y, Andersson U, Tracey KJ, Antoine DJ. Redox modification of cysteine residues regulates the cytokine activity of HMGB1. *Mol Med*. 2011 doi: 10.2119/molmed.2011.00389. [Epub ahead of print].
- [77]. Hreggvidsdottir HS, Ostberg T, Wähämaa H, Schierbeck H, Aveberger AC, Klevenvall L, Palmblad K, Ottosson L, Andersson U, Harris HE. The alarmin HMGB1 acts in synergy with endogenous and exogenous danger signals to promote inflammation. *J. Leukoc Biol*. 2009; 86:655–662. [PubMed: 19564572]

- [78]. Wähämaa H, Schierbeck H, Hreggvidsdottir HS, Palmblad K, Aveberger AC, Andersson U, Harris HE. High mobility group box protein 1 in complex with lipopolysaccharide or IL-1 promotes an increased inflammatory phenotype in synovial fibroblasts. *Arthritis Res Ther*. 2011; 13:R136. [PubMed: 21871094]
- [79]. Pisetsky D. Cell death in the pathogenesis of immune-mediated diseases: the role of HMGB1 and DAMP-PAMP complexes. *Swiss Med Wkly*. 2011; 141:13256.
- [80]. Bell CW, Jiang W, Reich CF 3rd, Pisetsky DS. The extracellular release of HMGB1 during apoptotic cell death. *Am J Physiol Cell Physiol*. 2006; 291:C1318–C1325. [PubMed: 16855214]
- [81]. Kazama H, Ricci JE, Herndon JM, Hoppe G, Green DR, Ferguson TA. Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. *Immunity*. 2008; 29:21–32. [PubMed: 18631454]
- [82]. Urbanaviciute V, Furnrohr BG, Meister S, Munoz L, Heyder P, De marchis F, Bianchi ME, Kirschning C, Wagner H, Manfredi AA, Kalden JR, Schett G, Rovere-Querini P, Hermann M, Voll RE. Induction of inflammatory and immune responses by HMGB1-nucleosome complexes: implications for the pathogenesis of SLE. *J Exp Med*. 2008; 205:3007–3018. [PubMed: 19064698]
- [83]. Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, Meller S, Chamilos G, Sebasigari R, Ricceri V, Bassett R, Amuro H, Fukuhara S, Ito T, Liu Y-J, Gillet M. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci Transl Med*. 2011; 9:1–11.
- [84]. Lee J, Sohn JW, Zhang Y, Leong KW, Pisetsky D, Sullenger BA. Nucleic acid-binding polymers as anti-inflammatory agents. *Proc Natl Acad Sci U S A*. 2011; 108:14055–14060. [PubMed: 21844380]
- [85]. Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. *Annu Rev Immunol*. 2011; 29:139–162. [PubMed: 21219181]
- [86]. Xu J, Zhang X, Monestier M, Esmon NL, Esmon CT. Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury. *J Immunol*. 2011; 187:2626–2631. [PubMed: 21784973]
- [87]. Mortensen ES, Rekvig OP. Nephritogenic potential of anti-DNA antibodies against necrotic nucleosomes. *J Am Soc Nephrol*. 2009; 20:696–704. [PubMed: 19329762]
- [88]. Bianchi ME. HMGB1 loves company. *J Leukoc Biol*. 2009; 86:573–576. [PubMed: 19414536]

Highlights

- • Extracellular DNA can emanate from cells during apoptosis, necrosis and NETosis
- • DNA's immune activity depends on molecules that promote access to internal sensors
- • Anti-DNA form immune complexes that stimulate plasmacytoid dendritic cells
- • DNA can interact with HMGB1, a non-histone nuclear protein with alarmin activity

Figure 1.

The generation of extracellular DNA during apoptosis. The figure illustrates the various mechanism by which DNA can leave cells during apoptosis and form immune complexes with anti-DNA antibodies. During apoptosis, the nucleus fragments and condenses and some DNA migrates to the surface to enter blebs (small dark circles on the cell surface). During late apoptosis or secondary necrosis, DNA exits the dying cell in various forms. Thus, DNA may leave cells alone or in association with HMGB1. Even if DNA and HMGB1 leave the cell separately, they may associate in the extracellular space. DNA may also leave cells in the form of microparticles (the detached form of blebs) with DNA present either on the particle surface or accessible in the particle interior to antibodies because of particle permeability. The various sources of DNA can all form immune complexes with anti-DNA autoantibodies, although the size and resulting composition of the complexes will all differ, ranging from a simple complex of DNA and anti-DNA to large, multicomponent complexes assembled on a particle structure. Some aspects of this figure, while plausible, have not yet been experimentally verified.

Table 1

Conditions with Increased Extracellular DNA
SLE
Pulmonary infarction
Trauma
Malignancy
Pregnancy
Sepsis
Drug-induced liver injury

Table 2

Determinants of the Immune Activity of DNA
Base sequence (e.g., CpG motifs)
Base methylation
Backbone chemistry
Protein interaction
Anti-DNA
LL37
HMGB1
Intracellular location