

# Crosstalk Between Apoptosis and Autophagy: Environmental Genotoxins, Infection, and Innate Immunity

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**ABSTRACT:** Autoimmune disorders constitute a major and growing health concern. However, the genetic and environmental factors that contribute to or exacerbate disease symptoms remain unclear. Type I interferons (IFNs) are known to break immune tolerance and be elevated in the serum of patients with autoimmune diseases such as lupus. Extensive work over the past decade has characterized the role of a protein termed stimulator of interferon genes, or STING, in mediating IFN expression and activation in response to cytosolic DNA and cyclic dinucleotides. Interestingly, this STING-dependent innate immune pathway both utilizes and is targeted by the cell's autophagic machinery. Given that aberrant interplay between the apoptotic and autophagic machineries contributes to deregulation of the STING-dependent pathway, IFN-regulated autoimmune phenotypes may be influenced by the combined exposure to environmental carcinogens and pathogenic microorganisms and viruses. This review therefore summarizes recent data regarding these important issues in the field of autoimmunity.

**KEYWORDS:** UV light, interferon, autoimmunity, apoptosis, autophagy, STING

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## Introduction

A balanced response to pathogens and other immune stimuli is important to maintain physiologic homeostasis. However, whereas infectious agents may cause organismal death if the immune response is not properly activated, hyperactivated or inappropriately timed immune responses can also lead to various pathologies. This issue is particularly relevant in autoimmune disorders such as lupus erythematosus (LE), in which aberrant immune signaling and autoantibody development are associated with a broad spectrum of negative outcomes ranging from skin rashes and dermal scarring to more deadly systemic effects that include nephrotoxicity. The molecular mechanisms of autoimmune disease pathogenesis remain largely unknown but are thought to involve both genetic and environmental contributions. Furthermore, recent data indicate that deregulation of cellular autophagic and apoptotic processes may contribute to immune disorders. Thus, this review discusses possible molecular mechanisms by which aberrant crosstalk between various intracellular signaling pathways associated with cellular responses to environmental DNA damaging agents and viral or microbial infection may exacerbate the phenotypes of autoimmunity.

## Type I Interferons and the Innate Immune Response

The type I interferons, which include IFN- $\alpha$  and IFN- $\beta$ , are important regulators of the innate immune response following infection. Interferons are cytokines that are secreted from viral- and microbe-infected cells and alert the immune system

to the presence of infection. Interferons act on the type I IFN receptor (IFNAR) on target cells to initiate intracellular signal transduction pathways that lead to the expression of antiviral and immunomodulatory genes that stimulate various immune cells, inhibit cell growth, and promote apoptosis following infection. Interestingly, alternations in IFN production and signaling are often found in patients with autoimmune disorders.<sup>1,2</sup>

Indeed, the identification of interferon activity in the serum of autoimmune patients initially suggested an aberrant role for IFN in the pathogenesis of autoimmunity.<sup>3</sup> Moreover, the levels of serum IFN are generally found to be correlated with disease activity and severity.<sup>4,5</sup> The notion that type I IFN may actively contribute to autoimmune phenotypes was based on the discovery that treatment of certain patients with type I IFN led to an increase in autoantibody production and to various autoimmune diseases,<sup>6–9</sup> and it has since been proposed that excess IFN could lead to a break in immune tolerance mechanisms through the activation of myeloid dendritic cells that subsequently stimulate autoreactive T cells.<sup>10–12</sup> The importance of IFN signaling to autoimmunity is also highlighted by the observation that most patients with systemic lupus erythematosus show signs of a so-called IFN gene signature in their blood.<sup>13–16</sup>

Understanding the mechanisms of IFN activation and production under normal and pathologic conditions may therefore reveal novel approaches to limit the progression and severity of



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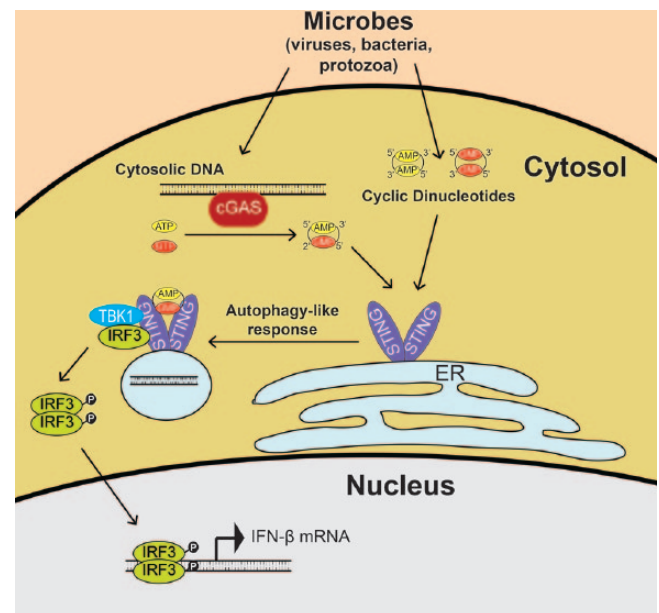
autoimmune diseases. Important insights into the mechanisms of IFN production have been made over the past decade with the discovery of signal transduction pathways that respond to pathogen-derived nucleic acids and other factors to induce IFN production.

### Control of IFN Expression and Innate Immunity by STING

The induction of IFNs requires the recognition of pathogen-associated molecular patterns (PAMPs) produced by viruses and microbes by specific pattern recognition receptors (PRRs) in the infected organism. This recognition can take place either outside the cell or within the cell and ultimately results in the activation of intracellular signaling pathways that induce IFN gene expression. Although a variety of biological macromolecules can act as PAMPs, including viral nucleic acids and bacterial cell wall components, the response of cells to pathogenic DNA and cyclic dinucleotides has attracted a great deal of attention over the past few years. Classical nucleic acid-sensing PRRs include Toll-like receptors (TLRs) that are present on the cell surface and within endosomes. However, TLR expression is typically restricted to specific cell types, such as plasmacytoid dendritic cells. Given that cells that do not express TLRs are also thought to be capable of responding to viral and microbial DNA to induce IFN production, it became clear that TLR-independent pathways must also exist in many diverse cell types to induce IFN and a subsequent innate immune response.

Indeed, the discovery of STING in 2008 (*stimulator of interferon genes*; also known as TMEM173, MITA, MYPS, and ERIS) as an endoplasmic reticulum-associated adaptor protein that facilitates IFN induction in response to nonself DNA was a major breakthrough in the field.<sup>17–19</sup> However, the mechanism of STING activation by DNA remained unresolved for a number of years. Although STING has some direct affinity for DNA,<sup>20</sup> microbe-derived 3',3'-cGAMP, c-di-AMP, and c-di-GMP had previously been shown to be ligands for STING and to induce IFN activation.<sup>21–28</sup> The important discovery in 2013 of the enzyme cyclic GMP-AMP synthase (cGAS),<sup>29</sup> which generates the cyclic dinucleotide 2',3'-cGAMP in response to DNA stimulation,<sup>21,22,30,31</sup> appeared to reconcile these findings. Thus, our current understanding of STING function is that it is activated by specific cyclic dinucleotides that are produced either directly by invading pathogens or indirectly by endogenous cGAS. Thus, the discovery of an endogenous ligand for STING, along with its corresponding native human biosynthetic enzyme, has provided important new insights into the mechanisms of innate immunity and generated great excitement in the field.<sup>32,33</sup>

The binding of cyclic dinucleotides to STING causes a conformational change in the protein that allows it to act as a scaffold or adaptor protein for the kinase TANK-binding kinase 1 (TBK1) to phosphorylate a transcription factor



**Figure 1.** Schematic of the stimulator of interferon genes (STING)-dependent innate immune response pathway. The infection of host cells by viruses and microbial pathogens results in the presence of nonself DNA or cyclic dinucleotides in the cytosol of the infected cells. The recognition of this DNA by the enzyme cyclic GMP-AMP synthase (cGAS) induces it to produce the cyclic dinucleotide cGAMP (cyclic GMP-AMP). Both cGAMP and other pathogen-derived cyclic dinucleotides bind STING, which alters the conformation of the protein and allows it to facilitate the phosphorylation of interferon regulatory factor 3 (IRF3) by TANK-binding kinase 1 (TBK1). Interferon regulatory factor 3 phosphorylation induces protein dimerization and entry into the nucleus, where it acts as a transcription factor to drive interferon (IFN) expression. ER indicates endoplasmic reticulum.

known as interferon regulatory factor 3 (IRF3),<sup>34</sup> which had previously been shown to be critical for IFN induction by cytosolic DNA.<sup>35</sup> This phosphorylation event induces the dimerization of IRF3 and allows it to enter the nucleus where it can function as a transcription factor to drive the expression of type I IFNs and other gene targets.<sup>35–37</sup> A schematic summarizing the STING-dependent production of IFN in response to viral and microbial DNA and cyclic dinucleotides is provided in Figure 1.

Interestingly, a recent observation that rare gain-of-function mutations in STING contribute to autoimmunity and autoinflammatory diseases in human populations<sup>38,39</sup> highlights the physiologic importance of STING in autoimmunity. Furthermore, these findings suggest that the identification and characterization of additional genetic and environmental factors that impact the STING pathway may provide new insights into autoimmune pathologies and provide novel approaches to control innate immune responses.

### Autophagy and STING-Dependent Innate Immunity

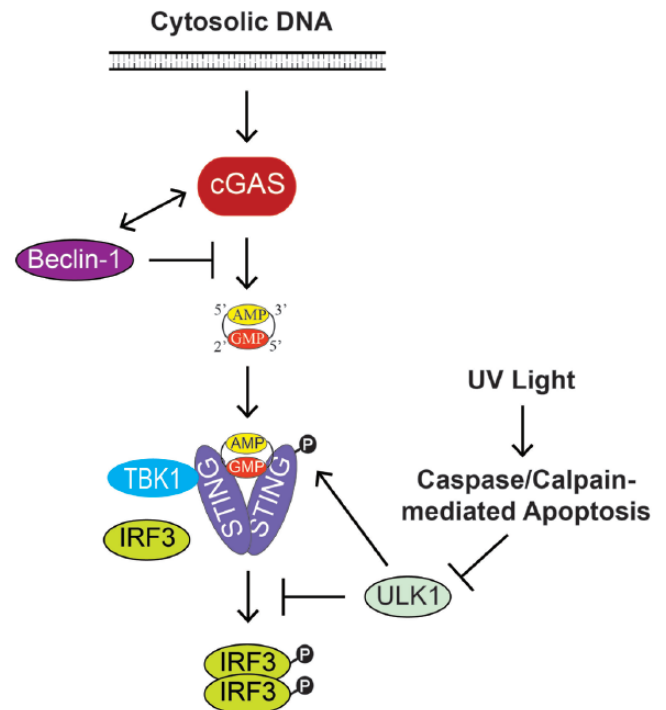
Although the cellular autophagic machinery plays well-recognized roles in breaking down damaged proteins and

organelles by sequestering and directing cargo to the lysosome, the same machinery is also required for host cells to cope with invading pathogens.<sup>40</sup> Indeed, viral infection and even synthetic DNA transfection have been shown to induce canonical biochemical read-outs of autophagy, such as LC3 lipidation, and to lead to the co-localization of cytosolic DNA with autophagic proteins.<sup>41–44</sup> Recent findings further show that this response to infection is tightly coordinated with the STING-dependent innate immune signaling pathway. For example, STING was shown to be required for the efficient ubiquitin-mediated autophagic clearance of *Mycobacterium tuberculosis* in macrophages,<sup>45</sup> and additional studies with the double-stranded DNA (dsDNA) genome viruses HSV-1 and human cytomegalovirus similarly demonstrated a role for STING in the induction of the autophagic response.<sup>46,47</sup> Moreover, the enzyme cGAS was demonstrated to be required to target cytosolic DNA from bacterial pathogens to the autophagy pathway.<sup>44,48–50</sup> Interestingly, other microbes can bypass this pathway by producing cyclic di-GMP that directly activates STING.<sup>49</sup>

The precise mechanism by which the cGAS-STING pathway engages the autophagic machinery to degrade viral and microbial DNA as well as cyclic dinucleotides remains to be better characterized. Interplay between the cGAS-STING innate immune response and the autophagic factors may improve the efficiency of viral pathogen recognition and removal from the infected cell. There is also evidence that autophagic proteins can directly interact with components of the cGAS-STING pathway and influence its downstream signaling. For example, the proautophagic protein Beclin-1, which plays important roles in the induction and maturation of the autophagosome, directly binds to cGAS to negatively regulate its activity and suppress cGAMP production.<sup>44,48</sup> Although this interaction is important for promoting efficient autophagy-mediated degradation of cytosolic pathogen DNA through release of the negative autophagy regulator Rubicon from the Beclin-1 complex, direct and negative regulation of cGAS activity by Beclin-1 also serves to prevent excessive or persistent immune stimulation by cGAS following dsDNA stimulation or HSV-1 infection.

Interestingly, the autophagy regulatory kinase UNC-51-like kinase 1 (ULK1) was reported to phosphorylate STING to suppress IRF3 activation.<sup>51</sup> This response occurred after the autophagy-dependent and STING-dependent delivery of TBK1 to endosomes or lysosomes and involved the dissociation of ULK1 from its repressor AMP-activated kinase (AMPK). This negative regulation of STING was shown to be dependent on cGAMP produced by cGAS, which indicates that cGAMP not only directly activates STING but also stimulates a negative feedback loop that shuts off STING activity to prevent the persistent induction of IFN.

Together, Beclin-1 and ULK1 provide 2 mechanisms by which the autophagic machinery may work to limit STING-dependent innate immune signaling in response to infection.



**Figure 2.** Interplay between autophagy, apoptosis, and the stimulator of interferon genes (STING) pathway. The canonical cytosolic DNA-cGAS-cGAMP-STING-IRF3 pathway is subject to regulation by autophagic and apoptotic signaling proteins. Beclin-1 directly interacts with cGAS to suppress cGAMP production. ULK1 phosphorylates STING to prevent IRF3 phosphorylation. However, at high DNA damage loads, UV light and related environmental agents activate caspase and calpain apoptotic signaling, which promotes ULK1 degradation and therefore de-represses the STING pathway. cGAS indicates cyclic GMP-AMP synthase; IRF3, interferon regulatory factor 3; TBK1, TANK-binding kinase 1; ULK1, UNC-51-like kinase 1.

A schematic summarizing this regulation of the cGAS-STING pathway is provided in Figure 2.

### Crosstalk Between Autophagy and Apoptosis

Autophagy and apoptosis are well recognized as being 2 important cellular processes that allow cells and organisms to cope with and respond to cellular damage induced by a variety of stressors. Whereas autophagy is thought to be the primary mechanism by which cells turnover damaged organelles and other smaller cellular constituents, apoptosis is utilized to get rid of whole cells. However, certain stimuli, such as nutrient deprivation or viral infection, can potentially lead to the activation of either pathway. Indeed, both processes can occur in the same cell, though often with different kinetics in which autophagy is utilized prior to the induction of apoptosis. Moreover, there are a variety of mechanisms by which the autophagic and apoptotic pathways can become intertwined to affect cell fate.<sup>52</sup>

Various cell stressors, including nutrient deprivation, can stimulate an autophagic response to allow cells to adapt to altered cellular or environmental conditions.<sup>53,54</sup> However, cells that are unable to cope with the stressor through autophagy

may ultimately undergo apoptosis. Under these conditions, it is expected that shutting off autophagic signaling may be important to conserve cellular resources for the process of apoptosis. Consistent with this notion, a variety of autophagic proteins, including ATG3, Beclin-1, and AMBRA1, have been shown to be targeted for caspase-mediated destruction during apoptosis.<sup>55–57</sup> Furthermore, the localization of the tumor suppressor protein p53 to the cytosol is associated with its interaction with FIP200 (FAK family kinase-interacting protein of 200 kDa), which blocks the activation of the ULK1-FIP200-ATG13 complex that is necessary for autophagosome formation.<sup>58,59</sup> Thus, there is clear evidence that apoptotic signaling can suppress autophagy under conditions of extreme cellular stress. As will be discussed below, this regulation may lead to transient alterations in cellular signaling responses following infection.

### Environmental Genotoxins and the Interplay between Apoptosis and Autophagy During an Innate Immune Response

Apoptosis can be induced by a wide variety of cellular conditions and environmental stressors. Interestingly, exposure to excessive amounts of UV wavelengths of sunlight has long been known to induce apoptosis in the skin and to exacerbate the symptoms of autoimmune disorders such as LE in susceptible individuals.<sup>60,61</sup> UV light induces photoproducts in DNA that interfere with DNA replication and transcription and therefore are potentially lethal to cells if the damage is not properly removed by the nucleotide excision repair system.<sup>62</sup> Indeed, clinical case studies have shown UV exposures to have serious consequences in individuals with a history of the autoimmune disorder lupus.<sup>63,64</sup>

The current paradigm for how UV-induced cell death in the skin may promote autoimmune disorders such as lupus is based on the notion that the defective clearance of UV-damaged, apoptotic keratinocytes leads to the development of antibodies against nuclear autoantigens that are released from dying cells.<sup>65–67</sup> Although this hypothesis has several attractive features, there have been criticisms that the methodologies used to measure cell death lack sufficient specificity.<sup>68–70</sup> Thus, the cell death-autoantigen release model remains to be fully tested and validated. Moreover, there are likely other mechanisms that may explain how lethal or even sublethal UV exposures could influence the UV-associated pathogenesis of lupus.

To examine the links between UV exposures and innate immune signaling further, a recent study using cultured keratinocytes and other human cells lines *in vitro* sought to clarify how UV radiation affected the STING-dependent innate immune signaling pathway.<sup>71</sup> Using dsDNA and specific cyclic dinucleotides (cGAMP, c-di-GMP) to mimic a viral or microbial infection, the irradiation of cells with UV light prior to, or soon after, DNA/cyclic dinucleotide transfection was found to potentiate the stimulatory effect of the

cytosolic DNA/cyclic dinucleotides on the STING pathway. Thus, increased IRF3 phosphorylation, dimerization, and nuclear entry were observed in cells containing cytosolic DNA or cyclic dinucleotides when the cells were exposed to UV radiation. Interestingly, the maximal effect of UV radiation occurred at doses that saturated the ability of cells to remove genomic damage by the nucleotide excision repair machinery. Moreover, the stimulation of the STING-dependent innate immune response was found to also occur with the DNA damaging UV mimetic and environmental carcinogen benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide, which indicates that other environmental agents of relevance to human health may also contribute to autoimmunity.

To uncover the mechanism of this phenomenon, potential roles for DNA repair intermediates and various DNA damage and cell stress response kinases were initially considered.<sup>71</sup> However, the results of these experiments indicated that some other aspect of the cellular response to DNA damage was responsible for potentiation of the STING pathway. Furthermore, it was noted that the kinetics of apoptotic signaling were closely correlated in time with a posttranslational loss in the expression of the autophagic proteins AMBRA1 and ULK1. Because ULK1 is a negative regulator of STING,<sup>51</sup> these results indicated that the stronger STING response that occurred following UV irradiation was likely due to the UV-dependent loss of ULK1 and a subsequent de-repression of STING function. Moreover, given that AMBRA1 was previously shown to directly affect ULK1 protein stability and to be controlled by a caspase-dependent and calpain-dependent pathway,<sup>72</sup> these various findings together suggested that UV light-induced apoptotic signaling and the corresponding loss of the STING negative regulator ULK1 were responsible for potentiating the cellular response to cytosolic DNA and cyclic dinucleotides (Figure 2). Consistent with this hypothesis, inhibiting caspase and calpain activation prevented both AMBRA1 and ULK1 loss and the ability of UV radiation to stimulate the cytosolic DNA-dependent activation of STING.<sup>71</sup>

Importantly, the timing of UV irradiation relative to the introduction of cytosolic DNA or cyclic dinucleotides was critical to the response, as exposure to repair-saturating UV doses more than 1 to 2 hours prior to or following the introduction of cytosolic DNA failed to potentiate the STING response.<sup>71</sup> Because ULK1 is required to initiate autophagosome formation, the premature degradation of ULK1 by UV-induced apoptotic signaling might be expected to prevent proper activation of the STING pathway. Similarly, the transient nature of the STING signaling pathway following the introduction of DNA or cyclic dinucleotides into the cytosol would mean that a delayed loss of ULK1 would occur too late to affect the rapid cellular response to STING ligands. Thus, these findings indicate that an optimal window of exposure to UV light and cytosolic DNA is important in determining how

apoptotic responses to UV affect the autophagic regulation of the STING pathway.

### Future Directions

The observation that apoptotic signaling induced by environmental agents such as UV light can affect the autophagic response to cytosolic DNA and cyclic dinucleotides provides a new mechanistic basis for exploring how environmental exposures influence autoimmune diseases. Human skin is constantly exposed to UV wavelengths of sunlight and to a variety of pathogens. Although both UV exposure and infection are known risk factors that contribute to autoimmune phenotypes in human patients, experimental models of autoimmunity have thus far failed to consider a combinatorial interaction of both factors in autoimmune disease pathogenesis. Thus, the results presented above suggest that aberrant crosstalk between the apoptotic and autophagic pathways in response to environmental carcinogens such as UV and viral and microbial infections may contribute to autoimmune disease phenotypes and should be explored in future studies.

### Author Contributions

MGK wrote the manuscript.

### Disclosures and Ethics

As a requirement of publication, author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality, and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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