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Intracellular Nucleic Acid Sensing Triggers Necroptosis through Synergistic Type I IFN and TNF Signaling

Michelle Brault, *† Tayla M. Olsen, * Jennifer Martinez, ‡ Daniel B. Stetson, * and Andrew Oberst* *  

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The sensing of viral nucleic acids within the cytosol is essential for the induction of innate immune responses following infection. However, this sensing occurs within cells that have already been infected. The death of infected cells can be beneficial to the host by eliminating the virus’s replicative niche and facilitating the release of inflammatory mediators. In this study, we show that sensing of intracellular DNA or RNA by cGAS-STING or RIG-I-MAVS, respectively, leads to activation of RIPK3 and necroptosis in bone marrow–derived macrophages. Notably, this requires signaling through both type I IFN and TNF receptors, revealing synergy between these pathways to induce cell death. Furthermore, we show that hyperactivation of STING in mice leads to a shock-like phenotype, the mortality of which requires activation of the necroptotic pathway and IFN and TNF cosignaling, demonstrating that necroptosis is one outcome of STING signaling in vivo.

The induction of programmed cell death is an important process for maintaining tissue homeostasis, as well as improving immune system function. Although billions of cells in our bodies die each day by apoptosis, the induction of lytic cell death programs during infection more readily instruct and aid our immune system. One such cell death program is programmed necrosis, or necroptosis, which is both morphologically and mechanistically distinct from apoptosis, and requires the activation of receptor interacting protein kinases 1 and 3 (RIPK1 and RIPK3) (1). Notably, RIPK3-deficient animals display increased susceptibility to a variety of viral infections (2–6), highlighting the role of this pathway in antiviral host defense. Furthermore, cells deficient in type I IFN signaling, an essential component of antiviral defense, fail to induce necroptosis following treatment with a variety of RIPK3-activating stimuli (7), suggesting that necroptosis may constitute an arm of the IFN-driven antiviral response.

The sensing of foreign nucleic acids is a key initiating event in the innate immune response to viral infection. The presence of DNA in the cytosol is one such signature of infection. In most cell types, cytosolic DNA activates the cGAS-STING pathway (8). Briefly, cGAS senses DNA and synthesizes the secondary messenger cyclic GMP-AMP (or cGAMP) (9), which binds directly to STING (10), leading to activation of TBK-1– and IRF3-dependent transcription of IFN-β (11). The production and signaling of type I IFN leads to the induction of hundreds of IFN-stimulated genes, inducing an antiviral state. As such, cGAS- or STING-deficient mice are highly susceptible to infection by various DNA viruses (12–15). Intriguingly, many of these viruses also lead to significant mortality in RIPK3-deficient animals (2, 3, 5, 16). A recent study found that murine gammaherpesvirus-68 (MHV68) could induce necroptosis in L929 cells, and that this response was prevented by knockdown of STING (17). However, given the complex cellular responses triggered by viral infection, coupled with the observation that L929 cells constitutively express and respond to TNF in an autocrine manner (18), the mechanistic and physiological links between STING signaling and necroptosis remain unclear.

In this study we show that the sensing of cytosolic DNA induces programmed necrosis through the activation of the cGAS-STING pathway in primary cells. Interestingly, robust activation of cell death by this pathway requires not only type I IFN signaling, but also an intact TNF signaling pathway. Furthermore, we show that RNA ligands acting through the sensors TLR3 or RIG-I also require both IFN and TNF signaling to induce necroptosis, highlighting the essential synergy between these signaling pathways in response to a variety of innate immune stimuli. Finally, we find that in vivo administration of a STING agonist leads to a fatal, shock-like inflammatory disease in mice. Mortality associated with this disease requires both TNF and IFN signaling, and is significantly rescued in animals lacking components of the necroptotic pathway. These results indicate that multiple nucleotide sensing pathways can trigger necroptosis through the synergistic action of IFN and TNF, and that this signaling can mediate lethal shock in mice.

Materials and Culture

Cell culture, transfection protocol, and reagents

Macrophages were differentiated for 7 d from whole bone marrow in RPMI 1640 (HyClone) with 10% CMG12-14 supernatant, 10% FBS (Gemini), 100 U/ml penicillin/streptomycin (HyClone), 2 mM glutamine (HyClone), 50 mM 2-ME, and 10 mM HEPES (HyClone). Macrophages were lifted with 4 mM EDTA in PBS, counted, and plated at 150,000 cells per well in 24-well plates for death assays. Primary mouse embryonic fibroblasts were cultured in DMEM (HyClone) with 10% FBS (Gemini), 100 U/ml penicillin/streptomycin (HyClone), 2 mM glutamine (HyClone), 10 mM HEPES (HyClone), and 1 mM sodium pyruvate (HyClone). Cell death
assays were performed using the IncuCyte bioimaging platform (Essen). Four images per well were captured, analyzed, and averaged. Death was measured by the incorporation of Sytox green (Life Technologies) and normalized to starting total cell count using Sytox green (Life Technologies). zVAD-FMK (SM Biologicals) was used at a concentration between 25 and 50 μM depending on application. Experiments where zVAD treatment alone exceeded 40% cell death were eliminated from analysis as background was deemed too high for reliable death data, although these experiments never showed trends not consistent with data shown. Transfections were done at a 1:1 ratio with Lipofectamine 2000 (Life Technologies) and 2 μg/ml calf thymus DNA (Sigma) or 2′,3′-cGAMP (InvivoGen), unless otherwise specified. IFN stimulatory DNA was annealed as previously described (11) and transfected at a concentration of 2 μg/ml. Poly(I:C) (EMD Millipore) was added directly to the media at 1 μg/ml. RIG-I ligand (5′-triphosphate RNA) was transcribed and purified as previously described (19) using a T7 MEGAscript kit (Ambion) and then transfected at a concentration of 1 μg/ml. High dose IFN A (PBL Assay Science) for cell death assay was 100 μM, based on the sp. act. of each lot. For LumineX panels, macrophages were transfected with each ligand and supernatants were collected after 6 h and frozen at −80°C until analyzed per the manufacturer’s guidelines.

**Results**

**Introduction of DNA into the cytosol can trigger necroptosis**

We sought to understand the cellular response to cytosolic DNA, using primary bone marrow–derived macrophages (BMDM) as an experimental system. We observed that introduction of DNA into the cytosol led to rapid and robust cell death in primary murine BMDM. As has been previously described (28), this cell death response was AIM2- and caspase-1–dependent and morphologically consistent with inflammasome-induced pyroptosis (Fig. 1A, 1B). Unexpectedly, however, upon addition of the caspase inhibitor zVAD-FMK, the macrophages underwent slower but equally robust cell death in response to DNA transfection (Fig. 1C). This cell death was morphologically consistent with programmed necrosis (Fig. 1B), a lytic cell death program dependent on activation of RIPK3. Indeed, we observed that RIPK3-deficient or MLKL-deficient macrophages were resistant to cell death in response to DNA transfection in combination with zVAD-FMK (Fig. 1C). All tested doses of cytosolic DNA triggered RIPK3-dependent death when combined with zVAD (Supplemental Fig. 1A). Furthermore, transfection with DNA led to phosphorylation of RIPK3 and MLKL (Fig. 1D), confirming that DNA stimulation can lead to necroptosis when caspases are suppressed.

Given the robust AIM2-dependent response observed upon introduction of cytosolic DNA alone, we next sought to determine whether components of the AIM2 inflammasome contributed to the cell death we observed upon DNA transfection combined with caspase inhibition. To assess this, we transfected DNA into macrophages lacking the inflammasome component AIM2, and measured their cell death response over time. Although pyroptosis could not occur in macrophages missing key inflammasome components, AIM2−/− and Caspase-1/11−/− cells could still undergo necroptosis in response to cytosolic DNA (Fig. 1E) in combination with caspase inhibition. These findings indicate that DNA stimulation can lead to necroptosis when caspases are suppressed.

**Cytosolic DNA triggers necroptosis via the cGAS-STING pathway**

In an effort to identify the pathway by which cytosolic DNA causes necroptosis, we tested macrophages lacking candidate DNA sensors. A proposed DNA sensor, DAI (encoded by Zbp1), has been shown to be important for the induction of necroptosis during herpesvirus infections (2). However, DAI-deficient macrophages were still able to undergo necroptosis following DNA transfection (Fig. 2A), implying that DAI is not required for the induction of...
DNA, or 2 short and long exposure following treatment of 5ZV AD. (Supplemental Fig. 1B), confirming that multiple types of cytosolic DNA can trigger necroptosis. Finally, to understand whether DNA ligands could induce necroptosis in additional cell types, primary mouse embryonic fibroblasts were treated with zVAD and transfected with calf thymus DNA or cGAMP. Similar to macrophages, these cells underwent necroptosis (Supplemental Fig. 1C). Together, these data indicate that activation of the cGAS-STING pathway can lead to necroptosis in multiple cell types.

**STING-dependent IFN production is necessary but not sufficient to induce necroptosis**

The canonical function of the cGAS-STING pathway is the production of type I IFN, and IFN treatment alone has been shown to induce necroptosis under certain conditions (31). We therefore tested whether autocrine or paracrine signaling by IFN produced upon stimulation of the cGAS-STING pathway was necessary for the induction of necroptosis. Following transfection with calf thymus DNA or cGAMP, we observed that IFN-αR1-deficient macrophages, which do not respond to IFN, were resistant to necroptosis (Fig. 3A, 3B). These results indicate IFN signaling is required to induce necroptosis following cGAS-STING stimulation, echoing results for other RIPK3-activating ligands like TNF, LPS, and poly(I:C) (7). Consistently with this finding, we observed that both STAT1 and STAT2, important signaling molecules downstream of IFN receptor stimulation, were required for cGAMP-induced necroptosis (Supplemental Fig. 1D). We next tested whether IFN signaling was sufficient to induce necroptosis in macrophages. We found that treatment of WT but not Rikp3-/- primary macrophages with recombinant IFN in combination with zVAD-FMK led to modest necroptotic cell death, and that unlike the robust and consistent necroptotic response observed upon STING activation, the magnitude of cell death triggered by recombinant IFN was highly variable between experiments (Fig. 3C, Supplemental Fig. 1E). Notably, however, the necroptotic response to even supraphysiological doses of recombinant IFN-α or IFN-β (Supplemental Fig. 1F) did not recapitulate that observed upon STING activation. Together, these data indicate that IFN signaling is necessary but not sufficient for the necroptotic response we observe upon activation of the cGAS-STING pathway.

**STING-dependent TNF production is required for induction of necroptosis**

Because IFN alone was unable to fully recapitulate the necroptotic response observed upon treatment with DNA or cGAMP, we hypothesized that other STING-dependent signaling might contribute to the activation of the necroptotic cell death program. To identify such a signal, we transfected WT macrophages as well as macrophages lacking STING with cGAMP and assessed cytokine production from these cells by Luminex. We observed that in WT macrophages, but not in Tmem173-/- cells, cGAMP treatment led to upregulation of TNF, a cytokine well characterized for its ability to induce programmed necrosis (Fig. 4A). This finding indicated that, as has been shown in other systems (32, 33), STING activation can lead to production of TNF as well as type I IFN.
We next sought to determine whether STING-dependent production of TNF was required for the cell death response to DNA. To test this, we transfected WT macrophages, as well as cells deficient for TNF, TNFR1 (Tnfrsf1a), or TNFR2 (Tnfrsf1b), with cGAMP. When caspases were suppressed, macrophages deficient in TNF or either of its receptors failed to undergo necroptosis after cGAMP or DNA stimulation (Fig. 4B, Supplemental Fig. 2A), demonstrating that TNF is essential for robust and efficient necroptosis following activation of the cGAS-STING pathway. As TNFR1 is well characterized as a driver of RIPK1 activation (34), we wanted to test whether the RIPK1 inhibitor, Necrostatin-1, would eliminate DNA- or cGAMP-induced necroptosis. Indeed, addition of Necrostatin-1 significantly reduced necroptosis following treatment with zVAD and either cytosolic DNA or cGAMP (Supplemental Fig. 2C), indicating that TNF-driven RIPK1 activation plays a critical role in DNA-induced necroptosis. Although TNFR1 drives RIPK1

![Figure 2](https://example.com/figure2.jpg)

**FIGURE 2.** Cytosolic DNA triggers necroptosis via the cGAS-STING pathway. (A) Kinetic cell death of WT or Zbp1<sup>−/−</sup> BMDM after treatment with 2 μg/ml cytosolic DNA and 50 μM pan-caspase inhibitor zVAD. (B) Kinetic cell death of WT, Mb21d1<sup>−/−</sup>, Tmem173<sup>−/−</sup> BMDM after treatment with 2 μg/ml cytosolic DNA and 50 μM pan-caspase inhibitor zVAD. (C) Kinetic cell death of WT, Ripk3<sup>−/−</sup>, or Mlkl<sup>−/−</sup> BMDM after treatment with 2 μg/ml cGAMP and 25 μM pan-caspase inhibitor zVAD. (D) Kinetic cell death of WT, Mb21d1<sup>−/−</sup>, or Tmem173<sup>−/−</sup> BMDM after treatment with 2 μg/ml cGAMP and 25 μM pan-caspase inhibitor zVAD.

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![Figure 3](https://example.com/figure3.jpg)

**FIGURE 3.** STING-dependent IFN production is necessary but not sufficient to induce necroptosis. (A) Kinetic cell death of WT or Ifnar1<sup>−/−</sup> BMDM after treatment with 2 μg/ml cytosolic DNA and 50 μM pan-caspase inhibitor zVAD. (B) Kinetic cell death of WT or Ifnar1<sup>−/−</sup> BMDM after treatment with 2 μg/ml cGAMP and 25 μM pan-caspase inhibitor zVAD. (C) Kinetic cell death of WT or Ripk3<sup>−/−</sup> BMDM after treatment with 100 U/ml recombinant IFN and 25 μM pan-caspase inhibitor zVAD.
activation, we sought to understand the observed requirement for TNFR2 in this setting. One known effect of TNFR2 signaling is the degradation of the cellular inhibitors of apoptosis (cIAPs), which restrict necrosome formation (35), and this derepression has been reported to be required in combination with RIPK1 activation by TNFR1 to allow necroptosis to proceed (36). To assess this, TNFR1- and TNFR2-deficient cells were treated with zV AD and the inhibitor of apoptosis protein antagonist BV6. We observed that cells deficient in TNFR2, but not TNFR1, were sensitized to necroptosis by addition of BV6 (Supplemental Fig. 2B), consistent with the idea that TNFR2 signaling in this setting acts to derepress necroptosis by degrading the cIAPs.

As some components of the necroptotic pathway are upregulated in response to IFN or TNF signaling, we sought to ensure that failure to induce necroptosis in TNF- or IFN-αR1−/− macrophages was not due to cell intrinsic defect in tonic protein levels. To determine if necroptotic pathway components were present at comparable levels in these cells, WT, TNF−/−, and Ifnar1−/− macrophages were blotted for RIPK1, caspase-8, RIPK3, and MLKL. Levels of these proteins were comparable between WT, TNF−/−, and Ifnar1−/− cells (Fig. 4C), indicating that the resistance of cells lacking TNF or IFN signaling does not arise from a failure to express components of the necrototic pathway. Given the profound resistance to STING-induced necroptosis observed in both TNF−/− and Ifnar1−/− cells, we sought to test whether lack of one of these signaling pathways affected the other. However, we found that upregulation of a panel of IFN-responsive genes by LPS, cGAMP, or recombinant IFN was intact in TNF−/− macrophages (Fig. 4D), indicating that the IFN pathway is intact in these cells. Furthermore, although we saw that cGAMP treatment led to upregulation of TNF message, recombinant IFN treatment failed to upregulate TNF in WT macrophages (Fig. 4E), indicating that IFN signaling alone is not sufficient to initiate TNF signaling. Together, these data indicate that TNF signaling in response to activation of the cGAS-STING pathway is required for necroptosis, and that TNF signaling acts in concert with, but independently from, the IFN pathway to trigger necroptosis in this setting.

Synergistic IFN and TNF signaling is also required for RIG-I– and TLR3-induced necroptosis

Coactivation of both IFN and TNF production is a motif observed downstream of multiple innate immune sensors. Because we observed synergistic signaling of IFN and TNF to trigger necroptosis upon activation of the cGAS-STING pathway, we tested whether similar synergy occurred downstream of other nucleotide sensing
pathways. To do this, we assessed the response to 5′-triphosphate RNA, a ligand for the cytosolic RNA sensor RIG-I, and to poly(I:C), a ligand for TLR3. Upon transfection of primary macrophages with 5′-triphosphate RNA or poly(I:C) in combination with caspase suppression, we observed robust RIPK3-dependent cell death (Fig. 5A). As expected, necroptosis induced by RIG-I ligand required MAVS, the downstream signaling partner of RIG-I (Supplemental Fig. 3A), confirming that this stimulus acted through the RIG-I-MAVS pathway. Analogously, and in accordance with previous studies (7, 37), we observed that poly(I:C) stimulation induced necroptosis in WT but not TLR3- or IFN-αR-deficient macrophages (Supplemental Fig. 3B, 3C). Furthermore, we saw that poly(I:C)-induced necroptosis did not require DAI (Zbp1) (Supplemental Fig. 3D), despite reports of DAI being a putative RNA sensor. When caspases were inhibited, RIG-I ligand similarly required IFN-αR1 to induce necroptosis (Fig. 5B), indicating that, like TLR signaling, RIG-I–induced necroptosis required an intact IFN pathway.

In order to determine whether these RNA ligands required TNF for efficient necroptosis, we stimulated WT and Tnf−/− macrophages with either 5′-triphosphate or poly(I:C). We found that TNF-deficient macrophages failed to undergo necroptosis following either poly(I:C) or RIG-I ligand stimulation (Fig. 5C), confirming that like DNA, RNA ligands require intact TNF signaling to induce necroptosis. Consistent with this, we saw that phosphorylation of RIPK3 was absent following poly(I:C) treatment in Ifnar1−/− macrophages, and significantly reduced in Tnf−/− cells (Fig. 5D), indicating a requirement for both TNF and IFN signaling downstream of poly(I:C) stimulation.

Finally, we sought to determine whether the moderate levels of cell death seen in WT macrophages following high dose IFN treatment were also dependent on the presence of TNF. To test this, we treated WT and TNF-deficient macrophages with a high dose of IFN and inhibited caspases. We found that Tnf−/− macrophages were completely resistant to IFN-induced necroptosis (Fig. 5E), indicating that even necroptosis induced by direct IFN stimulation requires TNF production and signaling. Taken together, these data indicate a broad requirement for synergy between IFN and TNF signaling for the induction of necroptosis downstream of multiple innate immune sensing pathways.

**STING agonists induce sterile shock in mice**

It has previously been established that activation of RIPK1 and RIPK3 contributes to the mortality associated with sterile shock models following administration of high doses of TNF (38). Because we observed a requirement for STING-dependent TNF production for the induction of necroptosis in vitro, we wondered whether systemic STING activation in vivo might also lead to RIPK3-dependent pathology. Notably, STING agonists are currently in clinical development as tumor immunotherapy agents, but potential adverse responses to systemic administration of these agents have not been studied. DMXAA is a potent activator of murine STING that has been shown to have antitumor properties in mouse models (39, 40). Notably, the action of DMXAA is associated with production of TNF and other inflammatory cytokines (41) and therefore provided an ideal tool to test if STING activation could induce RIPK3 activation in vivo. Consistent with its role as a STING agonist, we found that DMXAA, in combination with zVAD, induced robust necroptosis in primary macrophages in vitro in a STING- and RIPK3-dependent manner (Supplemental Fig. 4A), analogous to the effects of cGAMP. To assess the systemic response to STING activation, we administered DMXAA i.p. and monitored mice for clinical signs of shock, such as hypothermia, lethargy, ocular secretions, and the production of proinflammatory cytokines. We found that DMXAA induced significant mortality in C57BL/6J (B6/J WT) animals, whereas STING-deficient animals were completely resistant to mortality (Fig. 6A). Even more strikingly, STING-deficient mice were protected from all observable signs of shock, and serum collected from these mice lacked detectable levels of the proinflammatory cytokine IL-6 and TNF-α (Fig. 6A, Supplemental Fig. 4B–D). These findings demonstrate that systemic DMXAA administration causes shock-like symptoms, and that this response is wholly dependent on STING signaling.

**Systemic shock induced by STING activation engages TNF, IFN, and necroptotic signaling**

We next sought to test whether the sterile shock and mortality triggered by systemic STING activation in vivo involved engagement of...
of TNF and/or IFN signaling. To do this, we administered DMXAA to \( \text{Tnf}^{-/-} \) and \( \text{Ifnar1}^{-/-} \) animals. Whereas a majority of WT animals succumbed to disease within 24 h, both \( \text{Tnf}^{-/-} \) and \( \text{Ifnar1}^{-/-} \) animals were completely resistant to mortality following DMXAA injection (Fig. 6B). Furthermore, mice lacking TNF or IFNAR1 showed a reduction in the inflammatory marker IL-6 upon systemic STING activation, although this reached statistical significance only in the case of TNF knockout (Fig. 6B, Supplemental Fig. 4D).

Given the synergy observed between IFN and TNF signaling in vitro, together with our finding that both pathways are involved in STING-driven shock in vivo, we next tested whether engagement of necroptotic signaling plays a role in the pathology observed upon DMXAA administration. To do this, we administered DMXAA to animals lacking either MLKL or RIPK3. \( \text{Mkl}^{-/-} \) animals were also significantly rescued from the lethal effects of STING activation, although not completely resistant (Fig. 6C). RIPK3-deficient and heterozygous animals were significantly rescued from mortality, whereas DMXAA was uniformly lethal to their WT littermates (Fig. 6D). Interestingly, whereas C57BL/6J mice have been shown to be more susceptible to recombinant TNF injection than C57BL/6N mice (42), we found the opposite following DMXAA administration (Fig. 6). Notably, we found that serum from mice lacking RIPK3 or MLKL had levels of the proinflammatory cytokine IL-6 comparable to those observed in

![Figure 6](http://www.jimmunol.org/)
WT mice following DMXAA treatment (Fig. 6C, 6D), suggesting that although the pathological response to inflammatory signaling is attenuated in these mice, the inflammatory signals themselves remain. Together, our findings indicate that activation of the cGAS-STING pathway in vivo causes activation of both IFN and TNF signaling, and that synergy between these pathways engages RIPK3 and MLKL to cause lethal shock.

Discussion
The sensing of foreign nucleic acids within the cell is a key initiator of the innate immune response to invading pathogens, and it can trigger diverse outcomes including both transcriptional changes and cell death. We show that following DNA detection, the cGAS-STING pathway can trigger necroptosis in primary macrophages when caspases are suppressed. Notably, this cell death response requires STING-dependent production of both type I IFN and TNF, and the induction of necroptosis by STING activation involves reciprocal, synergistic signaling by these two pathways. Although a similar finding of STING-dependent death was noted in a recent study using infection of L929 cells (17), our study provides a more complete understanding of this phenomenon through the use of primary cells and purified ligands, and avoids the confounding fact that L929 cells constitutively produce and respond to TNF in an autocrine manner (18). In addition, as the type I IFN and TNF signaling pathways are both activated downstream of other ligands, we show that RNA detection through both the RIP-I-MAVS pathway and the TLR3 pathway can trigger RIPK3 activation via a similar mechanism when caspases are suppressed, again requiring synergistic signaling through IFN and TNF.

The cGAS-STING pathway has been shown to play a critical role in the response to numerous DNA virus infections, through the production of type I IFN and resulting antiviral state (8). Viruses such as vaccinia trigger STING activation (14) but also encode caspase inhibitors that have been shown to inhibit caspase-8 and lead to RIPK3 activation (43). Although STING-dependent IFN production by infected cells may lead to the induction of an antiviral state in neighboring cells by paracrine signaling, our data would suggest that it may also act in an autocrine manner to induce death of the infected cell itself, thereby eliminating the replicative niche of the virus. This implies that RIPK3 activation represents an arm of the IFN-driven antiviral response, triggered only when the cell is exposed to coincident IFN and TNF signaling, as well as viral caspase suppression. This requirement may allow for communication of the need for an antiviral response through paracrine signaling, and concurrent elimination of the infected cells themselves.

In addition to its antiviral role, recent work has established STING as a mediator of beneficial antitumor immune responses (44). DMXAA was a clinical failure because it activates only murine, but not human, STING (45). However, stabilized cyclic dinucleotide compounds that activate human STING are currently in clinical trials as tumor immunotherapy agents. These compounds require STING for their efficacy, and lead to the production of both TNF and IFN (40). Our data indicate that dose-limiting toxicity of these agents likely involves engagement of the necroptotic pathway. Although the long-term efficacy and toxicity of these compounds at clinically relevant doses has not yet been established, our findings indicate that modulation of the necroptotic pathway may help to alleviate any shock-like adverse reactions to these drugs. Conversely, it is unknown whether the antitumor effects of cyclic dinucleotides involve activation of RIPK3 downstream of STING signaling within the tumor microenvironment. It is possible that RIPK3 engagement and necroptosis contribute to the efficacy of STING agonists, as Ags derived from necrotic cells activate T cell responses more readily than those derived from apoptotic cells and might better inform a cellular immune response to tumors (46).

A surprising facet of our data is the finding that both TNF and IFN signaling are required for induction of necroptosis by stimulation of TLR3, a pathway in which the RHIM-containing adapter TRIF is able to directly engage the RIP kinases (37, 47). This requirement is not explained by TNF- or IFN-mediated induction of known components of this signaling pathway. Furthermore, we show that necroptosis triggered by IFN signaling in the absence of pathogen-associated ligands requires TNF signaling, despite a lack of TNF induction upon IFN treatment. Thus, at least in this setting, it appears that tonic levels of TNF signaling are required to maintain cells in a necroptosis-competent state. This state likely reflects a combination of RIPK1 activation via TNFR1, and control of cIAP levels via TNFR2. How TNF and IFN interface in this setting remains to be understood. Future work will assess the role of these two pathways in the induction of necroptosis in infection and coinfection models where both IFN and TNF signaling are critical for pathogen clearance.

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Disclosures
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