Removing the TREX1 Safety Net: Oxidized DNA Overcomes Immune Silencing by Exonuclease TREX1

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http://dx.doi.org/10.1016/j.immuni.2013.08.023

If DNA accumulates in the cytosol, it activates innate immunity via recently described DNA sensors. In this issue of Immunity, Gehrke et al. (2013) show that oxidized DNA is resistant to degradation by TREX1 and thus has heightened immunostimulatory capacity.

When DNA accumulates within the cytosol, it becomes immune stimulatory because of the activation of cytosolic DNA sensors that lead to induction of cytokines and type I interferons (IFNs). Such cytosolic sensing is thought to occur both in the context of pathogen sensing (for example of DNA viruses) and in response to mislocalized self-DNA and can be a trigger for pathological inflammation and autoimmunity (Paludan and Bowie, 2013). As such, cytosolic DNA has been perceived as both a pathogen-associated molecular pattern (PAMP) and a damage-associated molecular pattern (DAMP), the latter being defined as a mediator perceived as both a pathogen-associated molecular pattern (DAMP), the latter being defined as a mediator associated with the cellular necrosis of damaged cells. Initially the authors noticed that genomic DNA from cells killed in different ways had differential immunostimulatory capacity, such that DNA from cells killed by UV irradiation had an enhanced ability to cause IFN-α induction when transfected into DCs. Such UV-killed cells generated reactive oxygen species (ROS) and superoxide, which oxidized guanine residues within DNA leading to 8-hydroxyguanosine (8-OHG) adducts. They went on to show that 8-OHG-containing DNA was itself more immune stimulatory than unmodified DNA and that the amount of 8-OHG within DNA directly correlated with the ability of the DNA to elicit IFN-α from DCs. A number of cell-culture-based experiments then modeled immune scenarios whereby increased oxidation of DNA might lead to heightened IFN-α responses (Figure 1).

In one scenario, pathogen DNA was exposed to ROS (as might occur during phagocytosis of pathogens by macrophages), and this rendered it more immune stimulatory when transfected into DCs. Interestingly, when 8-OHG DNA was incubated with the cationic antimicrobial peptide LL-37, which is known to be generated from cells such as neutrophils during innate immune responses to pathogens, transfection was no longer required in order for the DCs to respond with IFN-α induction, whereas there was no such response to equal amounts of unmodified DNA in complex with LL-37 (Figure 1). LL-37 has previously been shown to facilitate the uptake of DNA into the cytosol of monocytes (Chamilos et al., 2012). During immune responses, neutrophils are known to expel their genomic DNA as neutrophil extracellular traps (NETs) and coupled to the oxidative bursts seen for activated neutrophils, Gehrke et al. (2013) suggested that there could be high concentrations of oxidized DNA at sites of infection. Because NETs also contain LL-37, this could represent a perfect storm for enhanced DNA sensing by surrounding cells at sites of infection rich in activated neutrophils. Gehrke et al. (2013) provided evidence for this scenario by showing that genomic DNA from activated human neutrophils had higher amounts of 8-OHG adducts and was more immunostimulatory in DCs compared to DNA from unactivated neutrophils. This work increases our understanding of how the immune system responds to DNA and also changes the current paradigm that cytosolic DNA responses are merely due to an accumulation of normal DNA within that compartment. Rather, oxidation can mark out either foreign or self-DNA as a signal of danger, thus defining 8-OHG DNA as a bona fide DAMP. The authors propose that oxidation of DNA confers an additional layer of information to distinguish danger and
damage from healthy states, thereby helping to avoid unwanted autoimmune reactions to self-DNA. In other words, oxidation of DNA increases the signal-to-noise ratio for DNA sensing by heightening the sensitivity of the DNA response circuits during immune reactions when such a response is appropriate.

The next question addressed by Gehrke et al. (2013) was why exactly oxidized DNA was more immune stimulatory than normal DNA in DCs. Conceivably, this could be due to 8-OHG DNA being taken up with greater efficiency than normal DNA into compartments where DNA is sensed or to a higher binding affinity of 8-OHG DNA for DNA sensors or to an increased resistance of 8-OHG DNA to nucleases. To address these possibilities, the investigators first confirmed both in vitro and in vivo that sensing of 8-OHG DNA was due to STING-depen-
dent cytosolic sensing and not Toll-like receptor-9 (TLR9)-dependent endosomal sensing. Furthermore, cGAS and not DDX41 nor p204 (i.e., mouse IFI16) were required for the response, at least in immortalized mouse macrophages. Thus, a picture emerged that UV- or ROS-mediated damage of DNA potentiates immunorecognition of DNA via cGAS and STING, but because 8-OHG and normal DNA were found to have the same binding affinity for cGAS, cGAS did not preferentially respond to 8-OHG DNA per se.

Moreover, there was no differential uptake or cellular compartmentalization of normal and 8-OHG DNA. Thus they next turned to examine the potential role of differential stability that could be impacted by the contribution of nucleases.

Deoxyribonuclease I (DNase I) is found in the extracellular space and digests DNA at sites of high cell turnover. DNase II is in lysosomes and degrades DNA from engulfed apoptotic or necrotic cells, whereas DNase III (TREX1) is found on the endoplasmic reticulum (ER) and digests DNA in the cytosol, for example DNA that would otherwise accumulate because of reverse-transcribed DNA from endogenous retroviruses. All three DNases have been implicated in autoimmunity both in mouse models and in terms of DNase mutations associated with human disease (Hornung and Latz, 2010). Here, although DNase I and DNase II indiscriminately digested normal and 8-OHG DNA, degradation of 8-OHG-containing DNA by TREX1 was far slower compared to that of unmodified DNA. Also, the RAW macrophage cell line, which has very low TREX1, responded similarly to oxidized and normal DNA whereas reconstituting cells with exogenous TREX1 restored 8-OHG DNA discrimination and removal of TREX1 both in vitro and in vivo led to a breakdown in this discrimination. Hence, because of its resistance to TREX1, a greater amount of 8-OHG DNA accumulates in the cytosol compared to normal DNA, leading to activation of the cGAS pathway at a much lower threshold than for normal DNA (Figure 1). TREX1 was previously shown to be an essential negative regulator of intrinsic autoimmunity triggered by endogenous retroviruses (Stetson et al., 2008), so the work by Gehrke et al. (2013) here extends our appreciation of the predominant role of TREX1 in immune silencing of DNA during normal homeostasis.

DNA sensing has been implicated both in the initiation and exacerbation of LE (Nagata et al., 2010), and Gehrke et al. (2013) were able to demonstrate that enhanced sensing of 8-OHG DNA had relevance for this disease. In the MRL/lpr mouse model of LE, MRL/lpr but not control mice produced IFN-α in response to UV-damaged self, but not normal self, DNA. After UV exposure, LE patients can develop lupus skin lesions, rather than transient sunburn, and 8-OHG is a known marker of such lesions (Lunec et al., 1994). Of note, LL-37 is also present in such lesions. Thus, oxidative damage of self-DNA together with LL-37-driven access to the cGAS-STING pathway may contribute to the higher autoimmune reactivity in LE patients, and consistent with that Gehrke et al. (2013) found that
skin samples from patients with UV-induced LE lesions showed colocalization of 8-OHG and MxA (an IFN-α-induced gene product) in the epidermis. Compellingly, oxidized self-DNA but not normal DNA induced skin lesions in the lupus-prone mice.

Altogether, the paper by Gehrke et al. (2013) improves our understanding of how innate immune DNA-sensing mechanisms discriminate between danger and normal homeostasis and offers some tantalizing clues as to how DNA sensing might drive and exacerbate IFN-mediated autoimmunity. In the future it will be important to determine whether oxidation of DNA is utilized to heighten responses during sensing of pathogens, as well as sensing of damage, because it remains to be determined whether pathogen DNA gets marked by oxidation during a live infection. It will also be of interest to determine the activity of TREX1 in different cells in vivo and to discover whether some primary cells lack TREX1 and therefore may be sites where autoimmunity initiates more readily because of a lower threshold response to self-DNA. The work here emphasizes the importance of the safety net that TREX1 normally provides in preventing cytosolic DNA-mediated immune responses, a safety net that is removed during immune reactions by the generation of oxidized DNA.

REFERENCES

Autophagy Meets Phagocytosis

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http://dx.doi.org/10.1016/j.immuni.2013.08.027

Autophagy can degrade intracellular bacteria, but how this pathway contributes to phagocytosis is unclear. In this issue of *Immunity*, Bonilla et al. (2013) demonstrate an additional role for autophagy in *Mycobacterium tuberculosis* internalization by macrophages.

During autophagy (and macroautophagy), diverse substrates ranging from specific proteins to damaged organelles can be sequestered within a double-membrane vesicle (the autophagosome) and delivered to the lysosome for degradation. Targeting to the lysosome represents a primary threat to the survival of an internalized microbe, and the contribution of autophagy to this process has been most extensively examined during mycobacterial infection of macrophages. Pioneering experiments with the tuberculosis vaccine strain *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) demonstrated that induction of autophagy leads to engulfment of bacteria in autophagosomes and reduced viability in macrophages (Gutierrez et al., 2004). Subsequent studies have made related observations with *Mycobacterium tuberculosis* (Mtb) and have expanded the role of autophagy to include antigen presentation and processing of antimicrobial molecules (Figure 1; Alonso et al., 2007; Jagannath et al., 2009; Ponpuak et al., 2010). For this reason, manipulation of autophagy could complement other strategies in the fight against this devastating pathogen.

Although the emphasis has been on the fate of bacteria after internalization, the role of autophagy during phagocytosis has not been examined. Bonilla et al. (2013) utilize mice that lack Atg7 in the myeloid compartment (Atg7−/− mice) to now provide evidence that the internalization step is also subject to regulation by autophagy in an unexpected way. Consistent with the function of Atg7 directly upstream of autophagosome formation, the authors show that macrophages derived from Atg7−/− mice harbor a higher amount of BCG and Mtb after in vitro infection. Surprisingly, this increase in bacteria was seen as early as 1 hr after infection, reflecting enhanced binding and internalization rather than impaired degradation. Also, a higher proportion of macrophages containing bacteria can be found in Atg7−/− mice.