Removing the TREX1 safety net: oxidized DNA overcomes immune silencing by exonuclease TREX1.

Andrew G. Bowie¹,*

¹School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin2, Ireland.

*Correspondence: agbowie@tcd.ie

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 immune stimulatory capacity.

When DNA accumulates within the cytosol it becomes immune stimulatory due to 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 released from damaged cells that acts to stimulate inflammation or immunity (Zelenay and Reis e Sousa, 2013). Indeed, if the DNA of damaged cells is not properly cleared and accumulates in tissues, innate immunity is activated, leading to severe anemia, chronic arthritis and in some cases lupus erythematosus (LE), due to overproduction of cytokines and IFNs from
macrophages and dendritic cells (DCs, Nagata et al., 2010). In recent years there has been a huge increase in our knowledge of how cytosolic DNA sensing mechanisms operate, with DNA sensors such as IFI16 and DDX41 shown to operate via the now well-defined STING-TBK1-IRF3 signaling axis (Paludan and Bowie, 2013), and such knowledge has recently culminated in the defining in multiple papers in past 8 months of a brand new signaling pathway involving the generation of the endogenous STING activator 2’3’-cGAMP by the enzyme cGMP-AMP synthase (cGAS) (reviewed in Xiao and Fitzgerald, 2013). Although the mechanisms of cytosolic DNA sensing are becoming established, the physiological contexts whereby DNA would accumulate in the cytosol have been less clear, especially since intracellular nucleases such as 3’ repair exonuclease I (TREX1) would be expected to prevent accumulation. In this issue of Immunity, Gehrke et al. (2013) now provide insight into this issue by demonstrating that DNA that becomes oxidized during cell death and immune responses has a greater potential to stimulate type I IFN from monocytes than normal DNA, due to it being resistant to TREX1 degradation.

Initially the authors noticed that genomic DNA from cells killed in different ways had differential immune stimulatory 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-hydroxyguanine (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 anti-microbial 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 suggested there could be high concentrations of oxidized DNA at sites of infection. Since 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 provided evidence for this scenario by showing that genomic DNA from activated human neutrophils had higher amounts of 8-OHG adducts, and was more immune stimulatory 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 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-dependent cytosolic sensing, and not toll-like receptor-9 (TLR9)-dependent endosomal sensing. Furthermore, cGAS, and not DDX41 nor p204 (ie 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 the question remained, why 8-OHG and normal DNA were found to have the same binding affinity for cGAS, so 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 which 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, while DNase III (TREX1) is found on the endoplasmic reticulum (ER) and digests DNA in the cytosol, for example DNA that would otherwise accumulate due to 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 unmodified DNA. Also, the RAW macrophage cell line, which has very low TREX1, responded similarly to oxidized and normal DNA while 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 due to 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 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 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 found that skin samples from patients with UV-induced LE lesions showed co-localisation 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 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, since 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 due to 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 which is removed during immune reactions by the generation of oxidized DNA.

[Andrew-I was wondering if you could conclude this preview on the same light]
hearted note that is reflected in the title. Could you return to the motif of the safety net and tell the readers how removal of the safety net leads to an innate immune response? Have shuffled the final paragraph around to include the safety net motif at the end]

References


FIGURE 1. Mechanisms whereby oxidized DNA is generated and sensed leading to interferon induction. 8-hydroxyguanosine-containing DNA (8-OHG DNA, shown in red) is generated by oxidation of DNA in UV-treated cells and during neutrophil activation due to the release of both superoxide and genomic DNA-containing neutrophil extracellular traps (NETs). When such DNA accesses the cytosol of dendritic cells, it accumulates due to being resistant to degradation by TREX1, in contrast to an equivalent amount of normal DNA (shown in blue), which is degraded by TREX1 when present in the cytosol. The presence of the antimicrobial cationic peptide LL-37 allows the direct delivery of 8-OHG DNA into the cytosol without the need for transfection, and may have a role in delivering DNA into DCs in many physiological contexts. Accumulating intracellular TREX1-resistant 8-OHG DNA binds directly to the cytosolic DNA sensor cGAS, leading to the generation of the STING activator 2’,3’-cGAMP, and subsequent type I interferon induction (IFNα and/or IFNβ).
This image depicts a diagram illustrating the role of reactive oxygen species (ROS), 8-OH-guanine DNA (8-OHG DNA), and immune responses in the context of DNA damage.

- **Dying cell** releases 8-OHG DNA and ROS.
- **Activated neutrophil** produces superoxide NETs.
- **TREX1** is activated and facilitates the removal of DNA from the cell.
- **IFNα/β** production is regulated by STING, cGAS, cGAMP, TBK1, and IRF3.
- Normal DNA degradation and 8-OHG DNA accumulation are indicated as immune stimulatory or silent states.

The diagram highlights the complex interactions between DNA damage, cellular response, and immune signaling pathways.