

DNA Mismanagement Leads to Immune System Oversight

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Trex1, a major 3' DNA exonuclease in mammalian cells, has been thought to act primarily in DNA replication or repair. Surprisingly, the major phenotype resulting from Trex1 deficiency in humans and mice is a chronic inflammatory disease. In this issue, Yang et al. (2007) report that Trex1 deficiency causes chronic activation of the ATM-dependent DNA-damage checkpoint and accumulation of a discrete single-stranded DNA species in the cytoplasm, either of which could contribute to chronic inflammation.

Studies of DNA damage and DNA repair have recently yielded several intriguing connections to the immune response. To this we can add exciting recent findings that mutations in Trex1, the major 3' DNA exonuclease in cells, cause inflammatory myocarditis in mice and a complex disease in humans called Aicardi-Goutières syndrome (AGS), which is characterized in part by an autoimmune syndrome similar to systemic lupus erythematosus (SLE) (Crow et al., 2006). Just a few years ago, there would have been few candidate mechanisms to link a DNA-processing enzyme to immunity, but it is testimony to the pace of recent discoveries that numerous possibilities can now be considered (Figure 1).

A study by Yang et al. (2007) in this issue adds two intriguing new angles to this emerging story. First, the authors demonstrate that Trex1 deficiency results in constitutive activation of the ATM-dependent DNA-damage checkpoint, as indicated by destabilization of Chk2, a key transducer kinase in the pathway, and increased levels of the tumor suppressor p53 and the downstream CDK inhibitor p21. As a result, *Trex1*-deficient fibroblasts are impaired in the G1/S phase transition. Second, in cells lacking Trex a discrete single-stranded DNA (ssDNA) species of 60–65 bases was found to accumulate in the cytoplasm, where Trex normally resides.

The origin of this DNA species and whether it is responsible for activating the DNA-damage checkpoint remain to be definitively established. Equally unclear is whether either the DNA species or the activation of the DNA-damage checkpoint are responsible for the autoimmunity observed in *Trex1*-deficient animals. Yet there

are precedents suggesting both as possibilities.

Recent studies have drawn a connection between the DNA-damage response, tumorigenesis, and the innate immune response. Activation of the DNA-damage response by genotoxic agents, or the constitutive activation observed in many tumor cell

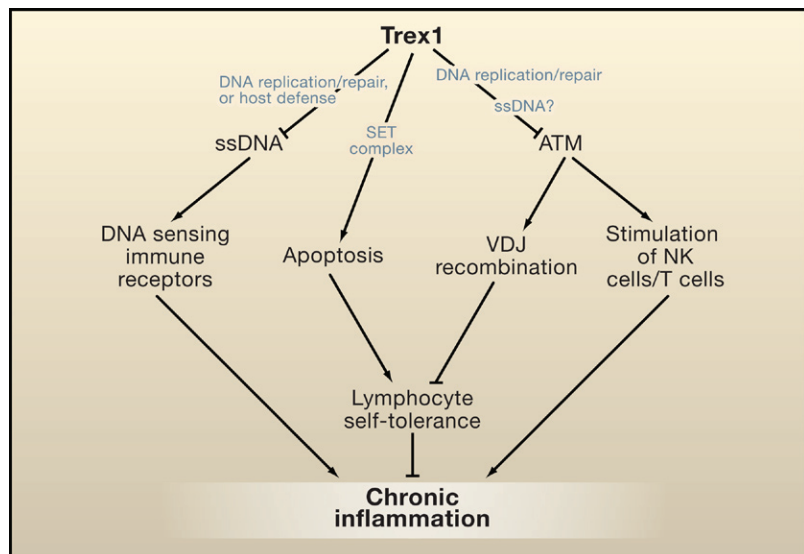


Figure 1. Inflammatory Disease and Trex1 Deficiency

Multiple mechanisms could account for the inflammatory disease in mice caused by Trex1 deficiency. Trex1 deficiency leads to the chronic activation of the cell-cycle checkpoint kinase ATM, which could induce expression of ligands that stimulate natural killer (NK) cells or T cells or could disturb T cell development thus interfering with the process that ensures lymphocyte self-tolerance. Lymphocyte self-tolerance might also be impaired in Trex1 mutant animals as a result of defective function of the Trex1- and granzyme-dependent SET complex. Alternatively, Trex1 may normally function in host defense to counter infection with DNA viruses or endogenous retroviruses, such that Trex1 deficiency leads to chronic activation of DNA-sensing receptors of the innate immune system.

lines, induces cell-surface expression of ligands that engage the NKG2D stimulatory receptor expressed by natural killer (NK) cells and certain T cell populations (Gasser et al., 2005). Because the DNA-damage response is activated early during the process of tumorigenesis, it has been proposed that induction of NKG2D ligands may serve as a barrier to tumorigenesis. NKG2D engagement by its ligands activates NK cells and T cells to kill target cells that express NKG2D ligands and to secrete inflammatory cytokines that may limit tumor development. Cytokine secretion could also underlie inflammatory disorders in cases where NKG2D ligands are inappropriately expressed. Interestingly, expression of NKG2D ligands by inflamed tissue and the NKG2D-dependent activation of immune cells that results have been linked to several chronic inflammatory diseases, such as rheumatoid arthritis, celiac disease, and type I diabetes (Ogasawara and Lanier, 2005). Thus, it is plausible that chronic activation of the DNA-damage response in *Trex1*-deficient cells causes inflammation by inducing expression of NKG2D ligands or other activators of the immune system. This possibility could be addressed by determining whether NKG2D function is necessary for disease in *Trex1*-deficient mice.

Our immune system encodes receptors that recognize specific microbial features (Akira et al., 2006). Some of these host receptors recognize viral RNA or DNA and are localized in the cytoplasm. Activation of these receptors, including a recently described DNA sensor called DAI (Takaoka et al., 2007), leads to the transcription of many genes, such as the antiviral cytokines known as type I interferons. Other receptors are localized either at the plasma membrane or in endosomes and sample the extracellular milieu for the presence of pathogens. One example is Toll-like receptor 9 (TLR9), an endosomal protein that recognizes unmethylated CpG motifs, a feature abundant in microbial genomes but also represented in mammalian DNA. TLR9 can bind pathogen DNA in endosomes after

pathogens are engulfed and partially degraded. Interestingly, it has been recently suggested that endosomal TLRs might also sample the intracellular milieu through autophagy. In this case, nucleic acids in the cytosol are engulfed into autophagosomes, vesicles capable of fusing with TLR-containing endosomes (Lee et al., 2007).

Self-DNA is normally restricted to the nucleus or the mitochondria, away from the DNA sensors. However, this is not the case under some pathological conditions, where activation of DNA sensors can be triggered in the absence of infection, leading to immune-associated pathology. Indeed, this is thought to occur in SLE, an autoimmune disease characterized by the presence of immune stimulatory complexes consisting of autoantibodies bound to DNA or to nucleic acid-protein complexes. Uptake of these immune complexes by dendritic cells and trafficking of the associated nucleic acid to TLR-containing endosomes is thought to amplify the disease by provoking secretion of type I interferons (Bacala et al., 2007).

The inflammatory syndrome associated with *Trex1* deficiency or observed in AGS patients is associated with elevated levels of type I interferons and accumulation of a ssDNA species in the cytoplasm. Mislocalization of nucleic acids to the cytoplasm or the extracellular milieu is thought to trigger antiviral immune responses and could be the culprit in causing the inflammatory disease in *Trex1*-deficient mice and AGS patients. Experiments testing whether these ssDNA molecules are subject to autophagy (for potential presentation to TLR9) or whether the known intracellular DNA sensors are activated in fibroblasts lacking *Trex1* might be highly informative.

Yang et al. (2007) attribute the ssDNA species seen in *Trex1*-deficient cells to byproducts of DNA replication, but there are other processes that might be involved in generating ssDNAs that are normally degraded by *Trex1*. *Trex1* is known to be a component of the SET complex, which includes the NM23-H1 endonuclease. NM23-H1 cooperates with

the 3' exonuclease activity of *Trex1* to destroy nicked double-stranded DNA (Chowdhury et al., 2006). The SET complex is implicated in apoptosis mediated by granzyme A, but it may have other functions in host defense. Thus, *Trex1* may be part of a complex that counters DNA viruses, or viruses with DNA intermediates, including endogenous retroviruses. Most endogenous retroviruses, which account for nearly 10% of the mammalian genome, have lost replication competence due to inactivating mutations, but several are still active. As part of their replicative strategy, they generate a DNA intermediate in the cytosol of their host cell. *Trex1* might function as part of the cellular machinery that degrades these intermediates. Incomplete degradation of such intermediates might result in the accumulation of the observed ssDNA species in *Trex1*-deficient cells.

The role of *Trex1* in granzyme A-mediated cell death suggests yet another mechanism underlying autoimmunity in *Trex1*-deficient animals. Granzyme A, along with perforin and other granzymes, is a component of cytotoxic granules released by cytotoxic T cells and NK cells. Defects in perforin- or granzyme-dependent cytotoxicity—as seen in perforin-deficient mice and patients with a disease called familial hemophagocytic lymphohistiocytosis—are also associated with autoimmunity (Janka, 2007). This may reflect the inability of mice to eliminate potentially autoreactive lymphocytes. Defective granzyme A action as a result of *Trex1* deficiency could similarly result in autoimmunity.

Finally, the chronic activation of the ATM protein (a cell-cycle checkpoint kinase) in cells lacking *Trex1* could promote autoimmunity by impacting lymphocyte development. The *ATM* gene was originally identified as the gene mutated in ataxia telangiectasia, a disease characterized by immune deficiency, progressive neurodegeneration, and sensitivity to irradiation. ATM-deficient mice, which exhibit a similar disorder, have partially impaired T cell development, owing to defects in the V-J recombina-

nation process (Vacchio et al., 2007). Thus, the chronic inflammatory phenotype of *Trex1*-deficient mice could be the result of alterations in T cell development resulting in impaired function of the T cells that regulate self-tolerance in the immune system. Chimera experiments to test whether the immune phenotype of *Trex1*-deficient mice is intrinsic to lymphocytes would be helpful in evaluating this and other related possibilities.

Thus, there are a startling number of ways that a defect in a DNA-processing enzyme could impact the immune response. It is particularly compelling that *Trex1*-deficient animals show no increase in mutation frequency or cancer incidence. This finding under-

scores the possibility that the primary biological role of *Trex1* lies not in DNA repair but in the regulation of immunity. It will be fascinating to see how the story resolves.

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tRNA Traffic Meets a Cell-Cycle Checkpoint

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The molecular pathways linking DNA-damage checkpoint proteins to cell-cycle progression remain largely unresolved. Findings by Ghavidel et al. (2007) reported in this issue suggest that tRNA trafficking and the transcription factor Gcn4 are key intermediates in the process by which yeast cells detect DNA damage and delay cell-cycle progression at the G1 to S phase transition.

DNA damage leads to the activation of a DNA-damage checkpoint that halts cell-cycle progression and alters DNA replication and repair to maintain genome stability (Kastan and Bartek, 2004). In general, DNA damage is initially detected by checkpoint proteins that once activated modify other proteins involved in either cell-cycle progression or DNA repair or replication itself. However, many molecular pathways connecting checkpoint proteins

to events in the cell cycle remain unresolved, although at least some checkpoint proteins alter the cell-cycle engine, for instance by regulating cyclins and cyclin-dependent kinases (CDKs). Ghavidel et al. (2007) now explore the mechanisms underlying the delay in the G1 to S phase transition (called START in the budding yeast *Saccharomyces cerevisiae*) in response to DNA damage. They provide surprising evidence that tRNA biogenesis and

trafficking are linked to cell-cycle progression by indirectly regulating the translation of the cyclin Cln2.

START in *S. cerevisiae*, analogous to the restriction point in mammalian cells, is the time in G1 when the cell monitors both growth status and genome integrity before committing to a round of cell division. Siede et al. (1993) were the first to observe that DNA damage in budding yeast causes a brief delay in the transition from G1 to S phase. Presumably