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NOD1 and NOD2: New Functions Linking Endoplasmic Reticulum Stress and Inflammation

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Although viruses have long been known to subvert the endoplasmic reticulum (ER) for their replication, recent work has shown that this strategy is also used by bacterial pathogens and parasites to promote their intracellular growth. The ensuing disruption of cellular processes triggers a condition known as ER stress, which activates the host cell's unfolded protein response (UPR) to restore homeostasis. Recent work has linked the UPR, in particular the arm of this response that depends on the ER-resident sensor IRE1, to innate immunity and inflammation. Surprisingly, two intracellular innate immune receptors, NOD1 and NOD2, previously shown to sense bacterial peptidoglycan, were found to transduce ER stress signals to elicit inflammation. Given the known roles of both ER stress and NOD2 in chronic inflammatory diseases, including inflammatory bowel disease and type 2 diabetes, this new link has important implications for understanding the basis for these pathologies.

Introduction

Stress to the cell's manufacturing machinery, the endoplasmic reticulum (ER), triggers a host responses known as the unfolded protein response (UPR). The UPR is designed to restore cellular homeostasis by transiently reducing protein translation (Ron, 2002) and inducing ER repair mechanisms, such as autophagy (Bernales *et al.*, 2006; Ogata *et al.*, 2006; Ding *et al.*, 2007). However, the UPR also gives rise to inflammatory responses, presumably because ER stress can be triggered by infection with a broad array of pathogens, including viruses, bacteria, and parasites. Many viruses cause ER stress by hijacking the cell's manufacturing machinery to produce envelope proteins [reviewed in (He, 2006; Smith, 2014)]. Some intracellular bacterial pathogens, including *Brucella* species and *Chlamydia* species, reside within vacuoles that can fuse with the ER membrane and use specific virulence factors to trigger the UPR [summarized in Celli and Tsolis (2015); Derre (2015)]. Extracellular bacterial pathogens, such as *Vibrio cholerae* or *Escherichia coli*, can produce toxins capable of triggering ER stress (Paton *et al.*, 2006; Cho *et al.*, 2011). The intracellular parasite *Toxoplasma gondii* induces ER stress by inducing oxidative damage to the mitochondria (Xu *et al.*, 2012). Thus, sensing pathogen-induced ER stress can enable the host to detect infection and induce antimicrobial responses to combat the intruding microbe [reviewed in Janssens *et al.* (2014)].

Detection of pathogens or pathogen-induced processes commonly involves pattern recognition receptors of the innate immune system. However, ER stress is sensed by three transmembrane receptors that are not commonly considered to be part of our immune system: ATF6 (activating transcription factor 6), PERK (protein kinase RNA-like endoplasmic reticulum kinase), and IRE1α (inositol-requiring enzyme 1α) (Fig. 1). Recent work from multiple groups has identified fascinating new connections between activation of the multifunctional IRE1 isoforms α and β and inflammation. IRE1's endonuclease function was recently recognized to transmit ER stress signals through two different mechanisms involving innate immune signaling molecules associated with mitochondria, which lead to proinflammatory cytokine release (Cho *et al.*, 2011; Bronner *et al.*, 2015).

Activation of a different activity of IRE1α, namely its kinase activity, initiates inflammatory responses through the recruitment of TRAF2 (TNF receptor-associated factor 2) (Urano *et al.*, 2000) to promote proinflammatory cytokine signaling, but the possibility that pattern recognition receptors could participate in this pathway has eluded the field until recently. Our recent work provides a new link between IRE1 activation during ER stress and inflammation that depends on IRE1's kinase activity. The results of this work suggest that TRAF2 interacts with the pattern recognition receptors NOD1 (nucleotide-binding oligomerization domain protein 1) and NOD2 to orchestrate this inflammatory branch of the UPR, which also requires the adaptor protein RIP2 (receptor-interacting serine/threonine-protein kinase 2) (Keestra-Gounder *et al.*, 2016). This pathway activates JNK (c-Jun N-terminal kinase), which triggers activation of nuclear factor kappa B (NF-κB) and expression of proinflammatory genes (Urano *et al.*, 2000) (Fig. 1).

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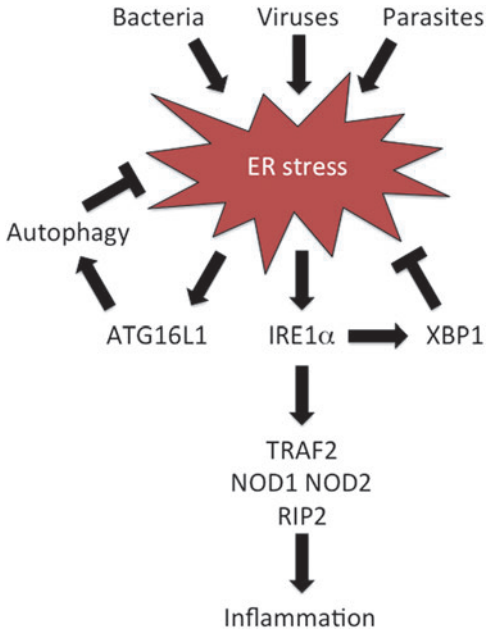

FIG. 1. Sensing of intracellular infection by the IRE1α branch of the endoplasmic reticulum stress response elicits inflammation through a pathway involving TRAF2, NOD1 and NOD2, and RIP2.

The canonical ligands for NOD1 and NOD2 are bacterial cell wall fragments, including diaminopimelic acid-type peptidoglycan fragments derived from Gram-negative bacteria and a muramyl dipeptide motif common to all bacteria, respectively (Chamaillard *et al.*, 2003; Girardin *et al.*, 2003a, 2003b; Inohara *et al.*, 2003). However, previous work suggests that NOD1 and RIP2 also respond to pathogen-induced processes, including an activation of small Rho GTPases by virulence factors of enteric pathogens (Boyer *et al.*, 2011; Keestra *et al.*, 2013). Furthermore, NOD2 has been implicated in the induction of innate immune responses during influenza virus infection (Sabbah *et al.*, 2009), which is difficult to explain by detection of bacterial cell wall fragments. The newly proposed function of NOD2 in orchestrating IRE1α-induced inflammatory responses (Keestra-Gounder *et al.*, 2016) now provides a plausible explanation, because influenza virus is known to trigger ER stress (Roberson *et al.*, 2012).

The involvement of NOD1 and NOD2 in orchestrating this inflammatory branch of the UPR has broad implications, because ER stress is linked to several inflammatory diseases, including diabetes (Montane *et al.*, 2014), atherosclerosis (McAlpine and Werstuck, 2013), and Crohn's disease (Kaser *et al.*, 2008). Crohn's disease is of particular interest in this context, because certain alleles of the human *NOD2* gene are major risk factors for developing this inflammatory disorder (Hampe *et al.*, 2001). NOD2 is expressed in monocytes (Ogura *et al.*, 2001) and Paneth cells in the crypt epithelium of the small intestine (Kobayashi *et al.*, 2005). Paneth cell function is altered in Crohn's disease, which leads to a decreased ileal synthesis of the antimicrobial peptides HD-5 (human defensin 5) and HD-6 (Wehkamp *et al.*, 2004). There is accumulating evidence that altered Paneth cell function in Crohn's disease is because of ER stress [summarized in Kaser and Blumberg (2014)]. Consistent with this idea, genetic variation in genes implicated in restoring ER homeostasis, including *XBP1* and *ATG16L1*, leads to increased ER stress in Paneth cells and elevates the risk of developing Crohn's disease (Kaser *et al.*, 2008; Deuring *et al.*, 2014). Previous attempts to link NOD2 to ER stress in Crohn's disease propose that stimulation with peptidoglycan fragments induces autophagy, an ER-stress repair mechanism that requires ATG16L1 (Cooney *et al.*, 2010; Homer *et al.*, 2010; Travassos *et al.*, 2010), thus suggesting that the illness may be triggered by bacterial factors. However, this model does not explain why viruses can be a potential trigger of human inflammatory bowel disease, as suggested by changes in the enteric virome in patients with Crohn's disease (Norman *et al.*, 2015). The finding that NOD2 orchestrates the proinflammatory branch of the UPR suggests that the trigger for Crohn's disease may instead be ER stress, which can be induced by bacteria, viruses, and/or parasites (Keestra-Gounder *et al.*, 2016).

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Disclosure Statement

No competing financial interests exist.

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