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EDITORIAL

Tumor Necrosis Factor-α–Induced Apoptosis in the Intestinal Epithelium due to Chronic Nuclear Factor Kappa B Signaling Is Mediated by Receptor Interacting Serine/Threonine Kinase 1

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is characterized by chronic intestinal inflammation and a breakdown of the epithelial barrier. Patients often display signs of sustained immune signaling downstream of inflammatory cytokines and excessive apoptotic and necrotic cell death of intestinal epithelial cells (IECs). Tumor necrosis factor (TNF)-α blockade, the most widely used treatment for moderate to severe IBD, has been shown to improve IEC viability and barrier function.[1] Yet, the fundamental question of how TNF-α induces cell death in the context of IBD is unanswered, because cell culture and animal experiments indicate that IECs are typically not susceptible to TNF-α.

The fate of cells exposed to TNF-α depends on the different protein complexes that can form on ligation of its receptor, TNF-α receptor 1 (TNFR1).[2] For example, receptor interacting serine/threonine kinase 1 (RIPK1) can interact with NEMO and IKKα/β to activate the master transcription factor nuclear factor kappa B (NF-κB) to induce the expression of cytokines and antiapoptotic genes. In this scenario, RIPK1 serves a scaffolding function, and its kinase activity is dispensable. If NEMO is inhibited after TNFR1 activation, then RIPK1 will be released to interact with FADD and caspase-8 to form a proapoptotic complex termed the *ripoptosome*, which requires the kinase activity of RIPK1. When apoptosis is inhibited after ripoptosome formation, RIPK1 phosphorylates and activates RIPK3 to mediate necroptosis, a form of programmed necrosis. Thus, NF-κB and its upstream cofactor NEMO synergistically prevent RIPK1 from activating cell death pathways on TNF-α stimulation. However, these prosurvival functions are difficult to reconcile with the observation that IBD is often associated with NF-κB activation.[3] In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Wong et al[4] elegantly combine a mouse model and an in vitro three-dimensional enteroid system to reveal a central role of RIPK1 in the setting of chronic NF-κB activation where TNF-α induces IEC death.

The authors first examine publicly available data sets and find that numerous NF-κB target genes are up-regulated in active IBD patients and decreased after anti–TNF-α treatment. They further demonstrate by immunohistochemistry that markers of NF-κB activation and apoptosis overlap within the intestinal epithelium of IBD patients, thereby supporting the premise that NF-κB signaling and IEC death occur together in the disease setting. In a previous study, the authors generated mice engineered to have chronic NF-κB activation through expression of a constitutively active *Ikkb* mutant in the intestinal epithelium. These IKKβ(EE)^{IEC} mice display TNF-α–dependent IEC death after inoculation with bacterial lipopolysaccharide.[4,5] In the current study, they build on this model by showing that injection with TNF-α is sufficient to induce hallmarks of IEC apoptosis in IKKβ(EE)^{IEC} mice, and that stimulation of enteroids derived from these mice with TNF-α in vitro induces apoptosis without the requirement of other exogenous microbial or immune signals. They next focus their attention on RIPK1 and RIPK3 because of their role in mediating downstream consequences of TNFR1 activation. Strikingly, pharmacologic or genetic inhibition of RIPK1 kinase activity, which is required for ripoptosome function but not for NF-κB activation, completely rescued the viability of IKKβ(EE) enteroids. In contrast, inhibition of RIPK3 failed to improve enteroid survival. Consistent with these results, the authors show that blocking RIPK1 but not RIPK3 promotes the survival of IKKβ(EE)^{IEC} mice and inhibits IEC death in vivo on challenge with lipopolysaccharide or TNF-α.

The detailed molecular crosstalk between NF-κB and RIPK1 activity in this setting remains unclear, but the authors perform several informative experiments that will guide future studies. Overexpression of the NF-κB target gene *Tnfaip3* (A20) has been shown to facilitate ripoptosome formation and RIPK1 kinase activation,[6] and they confirm that the expression of this gene is spontaneously increased in IKKβ(EE) IECs. However, replacing A20 with an inactive variant did not affect susceptibility to TNF-α–induced cell death, suggesting that additional factors may also contribute to the pathogenic consequences of chronic NF-κB signaling. Consistent with the observation that reactive oxygen species (ROS) participate in RIPK1-mediated cell death,[7,8] the authors find that the administration of a ROS scavenger blocked apoptosis in both IKKβ(EE)^{IEC} mice and enteroids. These experiments show that TNF-α–stimulated IKKβ(EE) IECs undergo apoptosis in a manner dependent on the ripoptosome and ROS and a potential role for A20 that requires further investigation.

In summary, Wong et al reveal a key requirement of the RIPK1 kinase activity for TNF-α–induced apoptosis in IECs sensitized by sustained NF-κB signaling as observed in IBD patients. Of note, genetic susceptibility can also contribute to altered NF-κB signaling and IEC death. Recent findings using animals and enteroids harboring mutations in IBD genes such as *Tnfaip3* and *Atg16l1* have shown that RIPK1 inhibition prevents TNF-α–induced IEC death.[9,10] Together with these studies, the findings by Wong et al suggest that

RIPK1 inhibitors currently being evaluated in the clinic represent a promising intervention strategy for IBD. Using markers of NF-κB signaling in IECs could be a way to identify the subset of patients most responsive to these drugs.

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Conflicts of interest
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