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EDITORIAL

Putting the Pieces Together: NOD-Like Receptor Protein 3 Inflammasome Priming and Activation in Barrett's Epithelial Cells

After insults such as injury caused by gastrointestinal reflux, the stratified squamous epithelium of the esophagus is replaced with glandular mucosa (Barrett's esophagus), which can be a mosaic of metaplastic columnar intestinal-like or gastric-like epithelium.[1] This Barrett's epithelium shows altered differentiation and is characterized morphologically by a sequence of dysplasia, from low-grade to high-grade and intestinal metaplasia, which ultimately may evolve to invasive cancer.

Endoscopic screening for Barrett's esophagus is widely practiced and patients often are selected for screening based on the presence of multiple well-established risk factors for Barrett's esophagus including chronic gastroesophageal reflux disease (GERD), older age (>50 y), male sex, white race, increased body mass index, intra-abdominal fat distribution, and hiatal hernia.[2,3] Although the exact pathophysiological mechanisms responsible for GERD remain unclear, studies have shown that mucosal immune and inflammatory responses, characterized by specific cytokine and chemokine profiles, may underlie the diverse esophageal phenotypes of GERD.[4] In GERD and Barrett's esophagus, an essential role has been ascribed to T cells in the initiation of inflammation in the esophagus, and a balance between T-cell responses and phenotype may play an important role in disease progression. Obesity is a chronic low-grade inflammatory state, fueled by adipose tissue–derived inflammatory mediators such as interleukin (IL)6, tumor necrosis factor (TNF)-α, and leptin, and highlights the link of inflammation and Barrett's esophagus.[5] In a study by the Mayo Clinic in 2012, it was reported that fat around the gastroesophageal junction and visceral fat were associated not only with Barrett's esophagus, but also with increased esophageal inflammation and high-grade dysplasia in subjects with Barrett's esophagus, independent of body mass index. Visceral fat therefore might promote esophageal metaplasia and dysplasia.[6] Proinflammatory cytokines (TNF-α, IL1β, IL6, and IL8) and chemokines (CXCL-1 and CXCL-2) have been shown to be induced in esophageal cells by exposure to acidified media (pH 4), especially in those cells lacking glutathione peroxidase 7.[7,8] Loss of glutathione peroxidase 7 expression is a critical step in promoting the TNF-α–induced activation of proinflammatory nuclear factor-κB signaling, a major player in GERD-associated Barrett's carcinogenesis.[8] Furthermore, up-regulation of ΔNp73 could be observed in esophageal tissues collected from patients with GERD and Barrett's metaplasia. ΔNp73 was induced by the proinflammatory cytokines, IL1β and TNF-α, and enhanced through exposure to bile acids.[9]

Toll-like receptor (TLR) 4 has been linked to inflammation-associated carcinogenesis and has been found to be increased significantly in Barrett's esophagus. The TLR ligand lipopolysaccharide (LPS) can activate nuclear factor-κB signaling and IL8 as well as cyclooxygenase-2 expression in Barrett's esophagus cell lines and ex vivo cultures, but not in normal squamous epithelium.[10] Microbial molecular products stimulate intestinal inflammation by activating TLRs and inflammasomes as part of the innate immune system. This system's contribution to esophageal inflammation is largely unknown. Gram-negative bacteria, which dominate the esophageal microbiome in reflux esophagitis, produce LPS. TLR4 signaling produces pro-IL1β, pro-IL18, and NOD-like receptor protein 3 (NLRP3), which prime the NLRP3 inflammasome.[11,12]

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Nadatani et al[13] showed that although normal squamous and Barrett's cells expressed similar levels of TLR4, LPS-induced TLR4 signaling, followed by increased TNF-α and IL8 secretion, could be observed only in Barrett's cells. Barrett's cells treated with LPS showed increased expression of pro-IL18, pro-IL1β, and NLRP3, and increased mitochondrial reactive oxygen species levels, caspase-1 activity, IL1β and IL18 secretion, and lactate dehydrogenase (LDH) release.

Inflammasomes are named for their pattern-recognition receptors (eg, NLRP1, NLRP3, NLRC4, AIM2), and the caspase-1 in the inflammasome complex can interact with these directly or indirectly.[11] In most cell types, inflammasome function requires 2 signals. The first signal induces the expression of pro-IL1β and pro-IL18, which primes the inflammasome for activation by a second signal. By using the specific TLR4 inhibitor TAK-242, the investigators showed a lack of LPS-induced phospho-p65 expression as well as a reduction in pro-IL18, pro-IL1β, and NLRP3 messenger RNA. Together this suggests that inflammasome priming in Barrett's cells is dependent on TLR4.

The second signal after priming is inflammasome complex formation and cleavage of procaspase-1 to its active form, which can be caused by a number of different stimuli including extracellular adenosine triphosphate (ATP). Treatment of Barrett's cells with LPS alone or in combination with exogenous ATP activated the inflammasome as measured by increased secretion of IL1β and IL18 and the release of LDH (an indicator of pyroptosis, an inflammatory form of induced cell death). This observation indicated that the NLRP3 inflammasome is activated by LPS in Barrett's cells. NLRP3 small interfering RNA abolished LPS-induced

increases in the secretion of IL1β and IL18 as well as the release of LDH. Because the inflammasome activity is regulated by caspase-1, the investigators showed that inhibition of caspase-1 using a specific inhibitor (Ac-YVAD-CHO) could prevent the secretion of IL1β and IL18 and the release of LDH. Next, using MitoSox Red (Waltham, MA) to measure superoxide produced in the mitochondria of Barrett's cells, the investigators analyzed the mitochondrial production of reactive oxygen species as a known regulator of NLRP3 inflammasome activity. LPS treatment caused a significant increase in MitoSox Red staining, which could be inhibited by treatment with a mitochondrial antioxidant, ultimately blocking the increased secretion of IL1β and IL18, as well as the release of LDH.

The work presented by Nadatani et al[13] applies the findings of earlier studies on the inflammasome in other cell types to compare and characterize the normal squamous epithelium vs the epithelium in Barrett's esophagus. Verbeek et al[10] described TLR4 as expressed in esophageal squamous cells and in Barrett's epithelial cells and reported that LPS caused a significant increase in the expression of cyclooxygenase-2 in biopsy specimens of Barrett's metaplasia that were cultured ex vivo. A study aiming to delineate the stromal response in GERD recently showed that treatment with acidified media and the TLR4 ligands LPS and HMGB1 increased subepithelial myofibroblasts and IL6 and IL8 secretion in primary cultures of these human stromal cells.[14] However, little is known about the role of inflammasomes in Barrett's esophagus. In a number of other cell types, NLRP3 inflammasome function requires 2 signals: a priming event such as LPS binding to TLR4 (inducing the expression of pro-IL1β and pro-IL18) and an activation signal (eg, extracellular ATP) that results in the secretion of the active forms of IL1β and IL18 and in the induction of pyroptosis. It has been shown in mouse macrophages that NLRP3 inflammasome function depends on LPS for priming and extracellular ATP for activation.[12,15] In contrast, in mouse dendritic cells, mouse astrocytes, and human monocytes, LPS alone (without exogenous ATP) can perform both functions, priming and activation, of the NLRP3 inflammasome.[12,16] The role of the NPLR3 inflammasome in Barrett's epithelial cells has been unclear, since it was unknown whether 1 or 2 signals would be required for function. Interestingly, Nadatani et al[13] showed that LPS alone (without exogenous ATP) can both prime and activate the NLRP3 inflammasome events, which might enable the predominantly gram-negative bacteria esophageal microbiome to contribute to inflammation-mediated esophageal malignancies.

Few studies have focused on the role of the esophageal microbiome in Barrett's esophagus to date.[17,18] By triggering molecular events that both prime and activate the NLRP3 inflammasome, LPS produced by the esophageal microbiome might contribute to inflammation-mediated carcinogenesis in Barrett's esophagus, a biologically significant event. This study suggests the intriguing possibility that manipulation of the esophageal microbiome could be a novel strategy to prevent cancer in Barrett's esophagus.

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