INTRODUCTION

Gastro-esophageal reflux disease (GERD) is a spectrum of disorders that occur when reflux of gastric contents into the esophagus causes symptoms and/or complications. The most typical symptoms of GERD are heartburn and regurgitation. Symptoms and complications occur due to contact of noxious components of the refluxate with the esophageal mucosa.

There is heterogeneity of GERD characteristics between patients with apparently similar amount of reflux. First, while 30% of patients with pathological esophageal acid exposure have visible esophageal mucosal injury at endoscopy (erosive esophagitis, EE) and up to 10% have Barrett’s esophagus, 60%-70% of patients with GERD have no visible macroscopic injury (non-erosive reflux disease, NERD). Second, symptoms do not accurately predict the severity of GERD and do not correlate well to endoscopic findings. Functional
heartburn patients experience GERD symptoms despite having no association with reflux events, while many patients with Barrett’s esophagus often do not present with heartburn despite having had years of pathological levels of acid reflux. Finally, although acid suppression with proton pump inhibitors (PPIs) is effective for many, over 30% of patients respond inadequately.\(^2\)\(^4\)\(^5\)

The components of the gastro-esophageal refluxate can vary, and systemic factors (e.g., psychological stress) will influence symptoms perception. However, a significant contribution to the pathogenesis of GERD and potentially the variability in disease expression may be explained at the mucosal level. This article discusses the current understanding of the mucosal pathogenesis of GERD.

## 2 | MUCOSAL PATHOGENESIS

Esophageal symptoms and/or injury develops when the contents of the gastro-esophageal refluxate interface with the mucosal epithelium, leading to a chain of events that can result in epithelial barrier disruption, activation of afferent nociceptive nerves, and inflammation. Although acid is believed to be the primary aggressor within the refluxate, other components including bile acids (from duodenal-gastro-esophageal reflux) and pepsin have also been implicated.

Our understanding of how these aggressive constituents lead to symptoms and injury in GERD has evolved over recent years, perhaps moving from a more simplistic view of barrier disruption and mucosal permeability to acid toward a more complex view, integrating this permeability change with epithelial cell and neuronal activation alongside cytokine-driven mucosal injury.

Here we look at the different aspects of esophageal mucosal pathogenesis of GERD and consider how the factors may interact according to our latest understanding.

### 2.1 | The epithelial barrier in reflux disease

Under normal circumstances, the threat imposed by noxious refluxate is met by mucosal defense mechanisms including local homeostatic repair induced by acid-sensing epithelial cells and neurons.\(^5\) The stratified squamous epithelium of the esophagus itself provides a tight protective barrier against luminal contents.\(^7\) Junctional complexes between esophageal epithelial cell membranes are composed of tight junctions, adherens junctions, and desmosomes which form a barrier against the diffusion of ions.\(^8\) Where macroscopic erosions are visible (i.e., EE) there is clear evidence of mucosal barrier deficiency. However, macroscopic erosions occur in a minority of patients with GERD. Nevertheless, it is apparent that there is evidence of mucosal barrier deficiency even in NERD, and it is likely that this plays a role in symptom pathogenesis.\(^9\) Seen under white light, but even more apparent under electron microscopy, epithelial dilated intercellular spaces (DIS) are seen in patients with NERD.\(^10\) As the term suggests, increased distance between esophageal mucosal epithelial cells is seen in DIS.

| Key Points |
| --- |
| • As the first line of defence against noxious gastric contents, the esophageal mucosa has a key role in disease and symptom pathogenesis in gastroesophageal reflux disease. |
| • Even in non-erosive reflux disease, the esophageal mucosal barrier is defective when compared to healthy controls. |
| • Symptoms occur after stimulation of afferent nerves, which can often be found in the mucosa. |
| • Mucosal inflammation is important in disease and symptom generation, and occurs due to a “cytokine sizzle” rather than due to direct caustic injury. |

Theoretically, DIS is a morphological representation of an impaired epithelial barrier whereby the increased space between neighboring epithelial cells enables noxious refluxate contents including H\(^+\) to access nerve endings more readily and stimulate acid-sensitive nociceptors.\(^11\) Experimental data demonstrate that DIS can be induced by acid exposure\(^12\) and DIS in GORD is resolved by therapy with proton pump inhibitors.\(^13\)

The morphological findings of DIS are reinforced by functional studies of epithelial permeability. Measurements of transepithelial resistance (TER) allow dynamic monitoring of changes in epithelial permeability over time. When human esophageal biopsies are placed in Ussing chambers and exposed to acidic and weakly acidic solutions a reduction in TER occurs (suggesting that there is increased paracellular permeability within the biopsy specimen, as would be expected in the presence of DIS). Furthermore, these studies suggested that biopsies from patients with GERD had a greater reduction in TER upon acid exposure than biopsies from control subjects, perhaps suggesting an inherent vulnerability to barrier dysfunction in GERD.\(^14\) However, the presence of transient DIS alone is unlikely sufficient for symptoms (perfusion-induced DIS in healthy volunteers does not induce symptoms.\(^12\) Symptom generation is likely to require a long-lasting DIS, allowing prolonged epithelial exposure to noxious stimuli.

Functional changes in esophageal mucosal permeability can be demonstrated in vivo using intraluminal impedance techniques. While impedance is used clinically in gastro-esophageal reflux measurements, in the collapsed esophagus the impedance electrodes lie in contact with the mucosa and are able to measure impedance to current flow within the mucosa (i.e., a low impedance suggests an easier ionic passage and thus increased mucosal permeability).\(^15\) Indeed, baseline mucosal impedance is significantly lower in patients with GERD compared to controls, and the value of the impedance correlates inversely with the severity of 24-hour esophageal acid exposure.\(^16\)\(^17\) Reinforcing the concept that increased epithelial permeability may contribute to symptom pathogenesis, low esophageal impedance is associated with increased...
sensitivity to perception of infused acid. These results collectively suggest that excessive acid reflux leads to an increase in epithelial permeability as evidenced by DIS, TER, and low mucosal impedance, and this may allow noxious luminal contents an easier passage to activate submucosal nerve endings and lead to heartburn symptom generation. 

Animal experiments have suggested that it is not just reflux exposure that may drive changes of epithelial permeability in GERD. Exposure of rats to acute stress was found to induce DIS and increase esophageal mucosal permeability to small molecules. In the mouse colon, exposure to acute stress altered expression of tight junction proteins, ZO-2, and occludin, while in the skin, acute stress stimulated mast cell degranulation and altered barrier function. The role of mast cells in stress-induced permeability has also been suggested in the esophagus, where the stress response mediator corticotropin-releasing hormone receptor subtype 2 expression was identified in the rat esophageal mucosa. Moreover, many GERD patients report a higher symptom burden with increased stress, and these findings suggest that the mechanism may involve not only increased central sensitization, but peripheral sensitization driven by permeability changes at the level of the esophageal mucosa.

The molecular events that account for defects in barrier function involve changes in the structure and function of adhesion molecules. Increased paracellular permeability, demonstrated by an enhanced fluorescein flux and reduced TER in NERD compared to controls, is associated with the disruption of the adherens junction encircling the epithelial cell membrane. Luminal acid-induced activation of a disintegrin and metalloproteinate (ADAM-10) and its subsequent cleavage of the adherens junction protein e-cadherin have been identified as major contributors of a disturbed junctional barrier in the esophageal epithelium. In line with the association between enhanced mucosal permeability and GERD pathophysiology, in vitro and animal studies have also described the dysregulation of tight junction molecules. A rat model of EE identified interleukin-6 as a mediator for the downregulation of desmosomes, the onset of DIS, and subsequently defective cell-cell contacts. Moreover, distinct patterns of localization and expression of tight junction proteins (Occludin, Claudin-1-4) have been described. While Claudin-3 expression decreases, Claudin-1 and Claudin-2 become upregulated in EE only. In addition, acid exposure reduced the expression of tight junction protein 1 (ZO-1) by human esophageal epithelial cells, whereas incubation with epithelial growth factor reversed this effect and inhibited barrier function impairment.

3 | MUCOSAL RESPONSE IN GERD

For years, the pathogenesis of esophageal mucosal inflammation in GERD was thought to proceed in a "top-down" fashion, with acid-peptic injury starting at the luminal surface. According to this traditional acid burn model of EE, the process begins with refluxed acid and pepsin damaging proteins in the tight and adherens junctional complexes between esophageal squamous cells, thus enabling hydrogen ions (i.e., acid) to penetrate the epithelium and kill the cells at the luminal surface. The death of surface epithelial cells is assumed to trigger an acute inflammatory response with the infiltration of granulocytes (neutrophils and eosinophils) into the epithelium and to induce a proliferative response in esophageal basal cells attempting to replace the damaged surface cells. The characteristic GERD histologic abnormalities of basal cell hyperplasia and elongation of the squamous papillae (projections of lamina propria with capillary vessels) were believed to be manifestations of this reflux injury-induced proliferative response. In addition to enabling the entry of hydrogen ions, the increased epithelial permeability caused by acid-peptic damage to tight and adherens junctional complexes also allows chloride ions to diffuse into the intercellular spaces. These ions generate an osmotic force that drags water from the lumen into the intercellular spaces, pushing the epithelial cells apart and thus creating DIS.

This traditional acid burn model of EE pathogenesis recently has been refuted, with several modern studies suggesting a new "cytokine sizzle" model in which the pathogenesis of reflux-induced esophageal mucosal inflammation may be cytokine-mediated rather than the result of an acid burn. The first of these studies was a histologic evaluation of rats in which reflux esophagitis was surgically induced by the creation of an esophagodudenostomy with preservation of the vagally innervated stomach, enabling gastric and duodenal contents to reflux freely into the esophagus. In this model, the first evidence of esophageal inflammation observed was a T-lymphocyte infiltration of the submucosa three days after the operation. This lymphocytic infiltrate reached the lamina propria by week 1 and the epithelial layer by week 3, and granulocyte infiltration was not a prominent feature until erosions developed around week 4. Hyperplasia of basal cells was evident by week 1, but surface cell death (i.e., erosion) was not detected until week 4, indicating that the death of surface cells was not the trigger for basal cell hyperplasia. DIS was not observed until week 1, and then at the bottom of the epithelium in the basal and suprabasal layers. Thus, the sequence of events (i.e., bottom-up mucosal injury) observed in this rat model of reflux esophagitis was exactly the opposite of what would be expected from the traditional acid burn hypothesis.

Since the initial esophageal abnormality observed in this rat model was immune cell infiltration in the absence of apparent surface injury, the investigators postulated that reflux-exposed esophageal squamous cells produced pro-inflammatory cytokines that were drawing immune cells to the tissue. In support of this notion, the investigators noted increased expression of interleukin (IL)-8, a potent pro-inflammatory cytokine, in and around squamous cells at the luminal surface of the rat esophagus shortly after esophagodudenostomy, prior to the appearance of significant epithelial inflammation. Accompanying experiments using cultures of human esophageal squamous cells, which are devoid of stromal and inflammatory cells, demonstrated that brief exposure to acidic...
bile salt solutions did not induce cell death, but rather caused the cells to secrete IL-8 and IL-1β, which in turn induced migration of lymphocytes and neutrophils. Thus, these findings in vitro support the concept that reflux-induced cytokine secretion by esophageal squamous cells is the initiator of reflux esophagitis (i.e., the cytokine sizzle).37

In further support of the cytokine sizzle model of reflux esophagitis, a number of clinical studies have found an association between pro-inflammatory cytokines and esophageal mucosal inflammation in GORD.38,39 For example, high levels of IL-8 expression have been found to correlate with the presence of intraepithelial neutrophils and disease severity in biopsy tissue specimens from patients with erosive and non-erosive esophagitis.40,41 In addition to IL-8, other pro-inflammatory cytokines that might contribute to the pathogenesis of reflux esophagitis have been found in esophageal biopsy specimens from GORD patients including IL-1β, IL-6, monocyte chemoattractant protein 1 (MCP-1), IL-33, tumor necrosis factor (TNF)-α and prostaglandin E2.38,40,42-47

To establish that the cytokine sizzle model of GERD pathogenesis suggested by animal studies was indeed applicable to humans, Dunbar et al. induced acute reflux esophagitis in 12 patients who had severe (Los Angeles grade C) EE healed with PPIs by stopping PPI therapy for 2 weeks.48 From baseline to 2 weeks off PPIs, GERD redeveloped quickly in these patients manifested by significant increases in their GERD-Health Related Quality of Life symptom scores and in their esophageal acid exposure times; endoscopic evidence of reflux esophagitis also was observed to develop in all 12 patients within 2 weeks.48 Esophageal biopsies (taken from areas without surface erosions) at 1 and 2 weeks off PPIs demonstrated increases in intraepithelial T lymphocytic infiltration; neutrophils and eosinophils were rare. Basal cell and papillary hyperplasia developed in areas without surface erosions, and DIS were observed in the basal and suprabasal areas of the epithelium.48

In this same study, Dunbar et al. assessed the esophageal mucosa with confocal laser endomicroscopy (CLE), which demonstrated significant increases in intercellular space width in the proximal and distal esophagus as esophagitis developed. Capillary width also increased significantly in both the proximal and distal esophagus by 2 weeks off PPIs. These data agree with earlier findings demonstrating that blood supply to the esophagus increases in response to luminal acid exposure.49,50 Patients in the acute esophagitis study received intravenous injections of fluorescein as part of the CLE protocol, and CLE images demonstrated increased fluorescein (leaked from esophageal capillary vessels) in DIS at 1 and 2 weeks off PPIs. This finding suggests that reflux-induced esophageal inflammation increases vascular permeability, and that fluid leakage from inflammation-damaged blood vessels contributes to DIS formation.48 Furthermore, blood vessels are located in the papillae and significant papillary hyperplasia was observed by 2 weeks off PPI therapy. Thus, vascular fluid leakage could explain why DIS are most frequently observed in the basal and suprabasal epithelial layers adjacent to hyperplastic papillae during mucosal inflammation.

3.1 Molecular basis of GORD-induced cytokine production

The molecular pathways linking reflux esophagitis to pro-inflammatory cytokine production involve the generation of reactive oxygen species (ROS), which stabilize and activate hypoxia-inducible factor (HIF)-2α. HIFs are heterodimeric transcription factors comprised of oxygen-regulated HIF-α subunits and a constitutively-expressed HIF-1β subunit.51 Hypoxia and ROS can stabilize HIFs, enabling them to accumulate in the cytoplasm and translocate to the nucleus where they induce transcription of their target genes, which include a number of pro-inflammatory cytokines such as IL-8 and IL-1β.52 Cultures of human esophageal squamous cells exposed to acidic bile salts in vitro produce ROS, stabilize HIF-2α, and secrete pro-inflammatory cytokines that trigger T-lymphocyte migration.53,54 Moreover, all of these acidic bile salt-induced effects can be blocked by inhibition of HIF-2α either through genetic knockdown or by administration of a highly selective small molecule pharmacologic inhibitor.54 Acidic bile salt-induced HIF-2α activation also leads to activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκ-B) transcription factor, demonstrating crosstalk between two key pro-inflammatory signaling pathways.54

Biopsy specimens of acute reflux esophagitis from patients in the aforementioned clinical study of Dunbar et al. exhibited significant increases in epithelial immunostaining for HIF-2α and phospho-p65 (an NFκ-B subunit), as well as increases in mRNA for a number of pro-inflammatory cytokines including IL-8, IL-1β, TNF-α, and cyclooxygenase-2.54 These biopsy specimens also demonstrated associations between levels of HIF-2α and levels of mRNA for these pro-inflammatory mediators, and between HIF-2α and phospho-p65, observations supporting the hypothesis that reflux esophagitis is initiated through a reflux-induced, cytokine-mediated process in which HIF-2α plays a central role.

4 ACID-SENSING RECEPTORS AND MUCOSAL INNERVATION

After esophageal epithelial exposure to acid, induction of inflammation and symptoms is likely, at least in part, to be transduced via acid-sensitive receptors expressed within the mucosa. Transient receptor potential vanilloid type-1 (TRPV1) is a non-selective Ca2+-permeant channel that has been widely proposed to be a candidate sensory transductor of reflux-induced symptoms since it can be activated by acid and is often involved in pain pathways.26,55-57 Neutrophils and other immune cells can also release protons from their exocytic granules and lysosomes into the microenvironment and this inflammation-induced microenvironment acidification conceivably could activate TRPV1 even in the absence of reflux episodes.65-63 TRPV1 expression has been demonstrated to be increased in biopsies taken from GERD patients compared to controls.54 Acid-activation of TRPV1 in cat esophageal mucosa has been shown to result in release of substance P and CGRP, strongly implicating a
nociceptive role. Similarly, TRPV1 activation in cultured human esophageal epithelial cells has been shown to result in the release of adenosine triphosphate (ATP), a neurotransmitter involved in pain signaling and inflammation. Moreover, a recent in vivo study demonstrated reduced acid-induced damage to the mucosal integrity of murine esophageal epithelium upon pharmacological blockade of TRPV1, suggesting a potential role for TRPV1 in mucosal barrier impairment in NERD. The protease-activated receptor 2 (PAR2): a receptor for trypsin, is reportedly upregulated in the esophageal mucosa of GORD patients, coupled with the expression of inflammatory mediators such as IL-8. The acid-induced activation of PAR2 has been shown to enhance ATP release from cultured esophageal epithelial cells, to increase TRPV1 phosphorylation, and to lead to heightened sensitivity to acid.

Other TRP channels may also be candidates for sensory transduction in the esophageal mucosa. Observations in humans have recently highlighted the sensory role of transient receptor potential melastatin 8 (TRPM8) channel, where cold water-induced esophageal pain, but infusion of menthol (TRPM8 agonist) into the esophagus of GORD patients induced heartburn. A study in the guinea pig esophagus also demonstrated expression of TRPM8 in jugular C fibers, suggesting a distinctive nociceptive role for TRPM8 in sensory transduction.

Acid-sensing ion channels (ASICs) are members of the voltage-insensitive, amiloride-sensitive degenerin family of cation channels. As their name suggests they can be activated by protons, and thus are candidates for a role in GERD pathogenesis. While ASIC1, ASIC2, and ASIC3 have been shown to be expressed in the rat esophageal mucosa, ASIC3 alone is expressed in the human esophageal epithelium. The importance of ASICs in nociception and inflammation is highlighted in transgenic animal studies which show changes in response to mucosal acid exposure, and their upregulation during gastrointestinal inflammation regulated by nerve growth factor and serotonin which directly interact with the ASIC3 gene promoter region and induce its transcription in peripheral sensory neurons.

In cultured human esophageal epithelial cells, the potentiating effect of trypsin on PAR2 activation was found to enhance weak acid-induced ATP release from esophageal epithelial cells through both TRPV1 and ASIC3. While the sensitization mechanisms of ASIC3 in esophageal epithelial cells are not well known, current studies suggest that the generation of heartburn perception and esophageal hypersensitivity is likely to involve the interaction of PAR2, TRPV1, and ASICs.

After gastric contents have injured or inflamed the esophageal mucosa, the ultimate pathway by which heartburn will be felt is via activation of nociceptive nerves by the receptors discussed above. The distribution and characterization of mucosal sensory nerves may play an important role in reflux hypersensitivity in NERD, and hyposensitivity in Barrett’s esophagus. In asymptomatic human subjects, a network of intra-mucosal nerves expressing calcitonin gene-related protein (CGRP) can be seen throughout the esophagus. In usual circumstances, these nerves lie deep in the esophageal mucosa in the distal esophagus, but lie very close to the luminal surface in the proximal esophagus (likely explaining the relative hypersensitivity of the proximal esophagus, in turn likely a protective feature to defend against aspiration). Interestingly, it has recently been shown that in NERD, but not erosive esophagitis, Barrett’s esophagus, or functional heartburn, the mucosal nerves lie close to the lumen in the distal esophagus of patients with non-erosive reflux. Recently these superficial mucosal nerves in NERD have been shown to strongly express TRPV1, suggesting that an acid-transduced nociceptive role of these nerves in heartburn perception is highly likely. In intriguingly these findings also suggest that DIS may play less of a causative role than was previously thought, since deep penetration of acid would not be required to activate these nerves. Deep nerves in EE, however, do not express TRPV1 and may express inflammatory receptors such as bradykinin receptors as in the colon and become activated by inflammatory mediators.

5 | TOWARD AN INTEGRATED MUCOSAL PATHOGENESIS OF GERD

Integrity of the epithelial barrier, and presence of acid-sensitive receptors and nerves, and mucosal inflammation are likely to play an overlapping, often interdependent, role in pathogenesis of esophageal pain and sensitivity. Acid may directly or indirectly, via the acidic microenvironment created by cytokine-induced inflammation, activate acid-sensitive receptors on esophageal mucosal afferent nerves and epithelial cells. The activation of acid-sensing receptors can cause release of neuropeptides such as CGRP and stimulate neurogenic inflammation and subsequent pain even in the absence of macroscopic injury. Nociceptive neurons can both be activated by signals from immune cells, and directly mediate inflammation by releasing neuropeptides upon activation by noxious stimuli (Figure 1). Release of inflammatory mediators such as IL-8, IL-1β, peripheral prostaglandin E2, and TNF-α is likely to directly activate or heighten the sensitization of peripheral afferent nerves as seen in animal studies of colonic tissue where previously silent visceral afferent nerves are activated by chemical and inflammatory mediators, and lead to continuous neuronal firing. Growth and location of nerves may also be regulated by inflammation, such as in the colon where trypsinase-positive mast cells release nerve growth factor during mucosal inflammation. Acid may upregulate both the expression and activation of PAR-2 in esophageal squamous cells. Activation of PAR-2 might increase permeability of the esophageal epithelium by causing the redistribution of tight junction proteins. Generation of inflammation within the mucosa is likely to cause intercellular edema via vascular fluid leakage, resulting in DIS and further increases in epithelial barrier permeability. Disruptions of esophageal epithelial barrier function in turn would enable further exposure of epithelial cells and mucosal nerves to the noxious components of refluxate. The cycle continues.

This paper demonstrates that much progress has been made in the understanding of the mucosal changes that develop after the
occurrence of excessive gastro-esophageal reflux. It also illustrates that more research is needed to fully understand the role and nature of the interaction between the gastro-esophageal refluxate and the mucosal epithelium, afferent nerves, and inflammatory pathways. Such research includes studies to understand the receptors by which the epithelial cells and nerves interact with the refluxate, to understand the mechanisms for afferent nerve migration and location, and to understand how inflammation interacts with nerve activation. As we further study the mucosal pathogenesis, we can hope to identify targets for treatment (likely topical therapies) to help a significant number of GORD patients including those with refractory symptoms.

**DISCLOSURE**

The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

AU and AN developed and wrote the scope of the review, DS and SS contributed to the revision of the manuscript, and RS and PW performed extensive manuscript revision. All authors reviewed the final version of the manuscript.

**ORCID**

Daniel Sifrim  
https://orcid.org/0000-0002-4894-0523

Philip Woodland  
https://orcid.org/0000-0003-2445-9724
REFERENCES

1. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology. 1995;109(2):601-610.

2. Vallik N. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. Am J Gastroenterol. 2006;101(8):1900-1920.

3. Lundell LR, Dent J, Bennett JR, et al. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. Gut. 1999;45(2):172-180.

4. Bredenoord AJ, Smout AJ. Refractory gastrooesophageal reflux disease. Eur J Gastro Hepatol. 2008;20(3):217-223.

5. Labenz J, Labenz G, Stephan D, Willeke F. Unzureichende symptomkontrolle unter Langzeittherapie mit PPI bei GERD – Fakt oder Fiktion? Insufficient symptom control under long-term treatment with PPI in GERD – fact or fiction? MMW - Fortschritte der Medizin. 2016;158(54):7-11.

6. Holzer P. Acid-sensing ion channels in gastrointestinal function. Neuropharmacology. Elsevier Ltd. 2015;94:72-79.

7. Tobey NA, Argote CM, Aywada MS, Vanegas XC, Orlando RC. Dilated intercellular spaces and shunt permeability in nonerosive acid-damaged esophageal epithelium. Am J Gastroenterol. 2004;99(1):13-22.

8. Farné R, Fornari F, Blondeau K, et al. Acid and weakly acidic solutions impair mucosal integrity of distal exposed and proximal non-exposed human oesophagus. Gut. 2010;59(2):164-169.

9. Calabrese C, Bortolotti M, Fabbri A, et al. Reversibility of GERD phenotypic changes in colonocytes following acute stress or activation of mast cells in mice: implications for delayed epithelial barrier dysfunction. Gut. 2006;55(5):655-661.

10. Choi E-H, Brown BE, Crumrine D, et al. Mechanisms by which psychologic stress alters cutaneous permeability barrier homeostasis and stratum corneum integrity. J Invest Dermatol. 2005;124(3):587-595.

11. Wu SV, Yuan PQ, Wang L, Peng YL, Chen CY, Taché Y. Identification and characterization of multiple corticotropin-releasing factor type 2 receptor isofoms in the rat esophagus. Endocrinology. 2007;148(4):1675-1687.

12. Naliboff BD, Mayer M, Fass R, et al. The effect of life stress on symptoms of heartburn. Psychosom Med. 2004;66(3):426-434.

13. Aziz Q, Fass R, Gyawali CP, Miwa H, Pandolfo JE, Zerbib F. Esophageal disorders. Gastroenterology. 2016;150(6):1368-1379.

14. Tack J, Pandolfo JE. Pathophysiology of gastroesophageal reflux disease. Gastroenterology. 2018;154(2):277-288.

15. Knowles CH, Aziz Q. Visceral hypersensitivity in non-erosive reflux disease. Gut. 2008;57(5):674-683.

16. Jovov B, Que J, Tobey NA, Djukic Z, Hogan BLM, Orlando RC. Role of e-cadherin in the pathogenesis of gastroesophageal reflux disease. Am J Gastroenterol. 2011;106(6):1039-1047.

17. Asaoka D, Miwa H, Hiraï S, et al. Altered localization and expression of tight-junction proteins in a rat model with chronic acid reflux esophagitis. J Gastroenterol. 2005;40(8):781-790.

18. Miwa H, oshima T, sakurai J, et al. Experimental oesophagitis in the rat is associated with decreased voluntary movement. Neurogastroenterol Motil. 2009;21(3):296-303.

19. Li FY, Li Y. Interleukin-6, desmosome and tight junction protein expression levels in reflux esophagitis-affected mucosa. World J Gastroenterol. 2009;15(29):3621-3630.

20. Mönkemüller K, Wex T, Kuester D, et al. Role of tight junction proteins in gastroesophageal reflux disease. BMC Gastroenterol. 2012;12:128.

21. Okuyama M, Fujiwara Y, Tanigawa T, Watanabe K. Roles of ZO-1 and epidermal growth factor in esophageal epithelial defense against acid. Digestion. 2007;75(2-3):135-141.

22. Vinkelnstein A, Wolf BS, Som ML, Marshak RH. Peptic esophagitis with duodenal or gastric ulcer. J Am Med Assoc. 1954;154(11):885-889.

23. Fiocca R, Mastracci L, Riddell R, et al. Development of consensus guidelines for the histologic recognition of microscopic esophagitis in patients with gastroesophageal reflux disease: the Esohisto project. Hum Pathol. 2010;41(2):223-231.

24. Tobey NA, Gambling TM, Vanegas XC, Carlson JL, Orlando RC. Physicochemical basis for dilated intercellular spaces in non-erosive acid-damaged rabbit esophageal epithelium. Dis Esophagus. 2008;21(8):757-764.

25. Souza RF, Huo X, Mittal V, et al. Gastroesophageal reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. Gastroenterology. 2009;137(5):1776-1784.

26. Hamaguchi M, Fujiiwa Y, Takashima T, et al. Increased expression of cytokines and adhesion molecules in rat chronic esophagitis. J Gastroenterol. 2011;46(4):287-295.

27. Hamaguchi M, Fujiwara Y, Tanigawa T, Watanabe K. Roles of ZO-1 and epidermal growth factor in esophageal epithelial defense against acid. Digestion. 2007;75(2-3):135-141.

28. Vinkelnstein A, Wolf BS, Som ML, Marshak RH. Peptic esophagitis with duodenal or gastric ulcer. J Am Med Assoc. 1954;154(11):885-889.

29. Fiocca R, Mastracci L, Riddell R, et al. Development of consensus guidelines for the histologic recognition of microscopic esophagitis in patients with gastroesophageal reflux disease: the Esohisto project. Hum Pathol. 2010;41(2):223-231.

30. Tobey NA, Gambling TM, Vanegas XC, Carlson JL, Orlando RC. Physicochemical basis for dilated intercellular spaces in non-erosive acid-damaged rabbit esophageal epithelium. Dis Esophagus. 2008;21(8):757-764.

31. Souza RF, Huo X, Mittal V, et al. Gastroesophageal reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. Gastroenterology. 2009;137(5):1776-1784.

32. Hamaguchi M, Fujiiwa Y, Takashima T, et al. Increased expression of cytokines and adhesion molecules in rat chronic esophagitis. J Gastroenterol. 2011;46(4):287-295.

33. Okuyama M, Fujiwara Y, Tanigawa T, Watanabe K. Roles of ZO-1 and epidermal growth factor in esophageal epithelial defense against acid. Digestion. 2007;75(2-3):135-141.

34. Winkelstein A, Wolf BS, Som ML, Marshak RH. Peptic esophagitis with duodenal or gastric ulcer. J Am Med Assoc. 1954;154(11):885-889.

35. Fiocca R, Mastracci L, Riddell R, et al. Development of consensus guidelines for the histologic recognition of microscopic esophagitis in patients with gastroesophageal reflux disease: the Esohisto project. Hum Pathol. 2010;41(2):223-231.

36. Tobey NA, Gambling TM, Vanegas XC, Carlson JL, Orlando RC. Physicochemical basis for dilated intercellular spaces in non-erosive acid-damaged rabbit esophageal epithelium. Dis Esophagus. 2008;21(8):757-764.

37. Souza RF, Huo X, Mittal V, et al. Gastroesophageal reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. Gastroenterology. 2009;137(5):1776-1784.

38. Hamaguchi M, Fujiiwa Y, Takashima T, et al. Increased expression of cytokines and adhesion molecules in rat chronic esophagitis. J Gastroenterol. 2011;46(4):287-295.

39. Okuyama M, Fujiwara Y, Tanigawa T, Watanabe K. Roles of ZO-1 and epidermal growth factor in esophageal epithelial defense against acid. Digestion. 2007;75(2-3):135-141.

40. Vinkelnstein A, Wolf BS, Som ML, Marshak RH. Peptic esophagitis with duodenal or gastric ulcer. J Am Med Assoc. 1954;154(11):885-889.

41. Winkelstein A, Wolf BS, Som ML, Marshak RH. Peptic esophagitis with duodenal or gastric ulcer. J Am Med Assoc. 1954;154(11):885-889.
hypersensitivity in nonerosive reflux disease. Gastroenterology. 2017;153(5):1230-1239.

81. “DDW ePosters”. [Online]. https://ddw.apprisor.org/epsAbstractDDW.cfm?id=3. Accessed May 16, 2020.

82. Brierley SM, Jones RCW, Xu L, Gebhart GF, Blackshaw LA. Activation of splanchnic and pelvic colonic afferents by bradykinin in mice. Neurogastroenterol Motil. 2005;17(6):854-862.

83. Sengupta JN, Gebhart GF. Characterization of mechanosensitive pelvic nerve afferent fibers innervating the colon of the rat. J Neurophysiol. 1994;71(6):2046-2060.

84. Iyengar S, Ossipov MH, Johnson KW. The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine. Pain. 2017;158(4):543-559. Lippincott Williams and Wilkins.

85. Zelenka M, Schäfers M, Sommer C. Intraneural injection of interleukin-1β and tumor necrosis factor-alpha into rat sciatic nerve at physiological doses induces signs of neuropathic pain. Pain. 2005;116(3):257-263.

86. St-Jacques B, Ma W. Peripheral prostaglandin E2 prolongs the sensitization of nociceptive dorsal root ganglion neurons possibly by facilitating the synthesis and anterograde axonal trafficking of EP4 receptors. Exp Neurol. 2014;261:354-366.

87. Peiris M, Bulmer DC, Baker MD, et al. Human visceral afferent recordings: preliminary report. Gut. 2011;60(2):204-208.

88. Hockley JRF, Barker KH, Taylor TS, et al. Acid and inflammatory sensitisation of naked mole-rat colonic afferent nerves. Mol Pain. 2020;16. https://doi.org/10.1177/1744806920903150

89. Dothel G, Barbaro MR, Boudin H, et al. Nerve fiber outgrowth is increased in the intestinal mucosa of patients with irritable bowel syndrome. Gastroenterology. 2015;148(5):1002-1011.e4.

90. Tobey NA, Carson JL, Alkiew RA, Orlando RC. Dilated intercellular spaces: a morphological feature of acid reflux-damaged human esophageal epithelium. Gastroenterology. 1996;111(5):1200-1205.

How to cite this article: Ustaoglu A, Nguyen A, Spechler S, Sifrim D, Souza R, Woodland P. Mucosal pathogenesis in gastro-esophageal reflux disease. Neurogastroenterology & Motility. 2020;32:e14022. https://doi.org/10.1111/nmo.14022