Gastroesophageal reflux disease: Update on inflammation and symptom perception

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Gastroesophageal reflux disease (GERD) is a common disorder in Western countries, with a significant impact on quality of life and healthcare costs. The mechanisms involved in the pathogenesis of symptoms remain to be fully elucidated. GERD symptoms and complications may result from a multifactorial mechanism, in which acid and acid-pepsin are the important noxious factors involved. Prolonged contact of the esophageal mucosa with the refluxed content, probably caused by a defective anti-reflux barrier and luminal clearance mechanisms, would appear to be responsible for macroscopically detectable injury to the esophageal squamous epithelium. Receptors on acid-sensitive nerve endings may play a role in nociception and esophageal sensitivity, as suggested in animal models of chronic acid exposure. Meanwhile, specific cytokine and chemokine profiles would appear to underlie the various esophageal phenotypes of GERD, explaining, in part, the genesis of esophagitis in a subset of patients. Despite these findings, which show a significant production of inflammatory mediators and neurotransmitters in the pathogenesis of GERD, the relationship between the hypersensitivity and esophageal inflammation is not clear. Moreover, the large majority of GERD patients (up to 70%) do not develop esophageal erosions, a variant of the condition called non-erosive reflux disease. This summary aims to explore the inflammatory pathway involved in GERD pathogenesis, to better understand the possible distinction between erosive and non-erosive reflux disease patients and to provide new therapeutic approaches.

Key words: Gastroesophageal reflux disease; Mucosal inflammation; Heartburn; Esophagitis; Hypersensitivity

Core tip: The present study aimed to explore the mechanisms involved in the pathogenesis of gastroesophageal reflux disease (GERD) symptoms and complications, which remain to be fully elucidated. Despite recent evidence confirming the important production of inflammatory mediators and neurotransmitters in the pathogenesis of GERD, the interplay between esophageal inflammation and hypersensitivity is not clear. Based on the literature and on personal experimental studies, this paper attempts to summarize the evidence concerning the inflammatory pathway involved in GERD pathogenesis, to better define the possible distinction between erosive and non-erosive reflux disease patients and to provide new therapeutic approaches.
INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common disorder in Western countries, with 10%-30% of the individuals affected every week by symptoms that have a significant impact on quality of life and healthcare costs\[^8\]\. In GERD, the mechanisms involved in the pathogenesis of heartburn and chest pain remain to be completely elucidated; however, acid and pepsin can induce macroscopically detectable injury to the esophageal squamous epithelium\[^9\]\. Injured or inflamed tissues release inflammatory mediators that can be detected by the immune system. Moreover, in response to these chemical insults, endothelial cells produce adhesion molecules, which recruit and activate leukocytes, thus mediating inflammatory conditions\[^10\]\. In an experimental model of reflux esophagitis and in patients affected by GERD, some of the mediators considered to be critical in the etiology of esophagitis are classic inflammatory products, such as prostanooids and reactive oxygen species (ROS)\[^11\]\. Several studies have also suggested that the mucosal immune and inflammatory responses, characterized by specific cytokine and chemokine profiles, may underlie the various esophageal phenotypes of GERD\[^12\]\. Indeed, the large majority of GERD patients (up to 70%) do not develop esophageal erosions, a variant of the condition called non-erosive reflux disease (NERD)\[^13\]\. Although several studies report the progression of NERD to erosive esophagitis (erosive reflux disease, ERD), structural and functional characteristics differentiate these two important GERD groups: NERD patients usually have a normal lower esophageal sphincter resting pressure, minimal esophageal body motility abnormalities, low esophageal acid exposure profile and minimal night-time esophageal acid exposure\[^14,15\]\. The symptom response, in NERD patients, to acid suppressive therapy is lower than that in patients with ERD\[^16\]\. In fact, in these patients, esophageal visceral hypersensitivity, sustained esophageal contractions or impaired tissue resistance, have been identified as possible mechanisms responsible for reflux symptoms and proton pump inhibitor (PPI) resistance\[^17-19\]\. This brief summary focuses on the inflammatory pathway involved in the pathogenesis of GERD, to better understand the distinction between ERD and NERD patients and, thus, to provide better therapeutic approaches.

DAMAGE INDUCED BY REFLUXATE

GERD is a complex disorder with the potential for developing esophagitis, esophageal strictures and Barrett’s esophagus\[^20\]\. GERD symptoms and complications may result from a multifactorial mechanism in which acid and acid-pepsin are the important noxious factors involved. Prolonged contact of the esophageal mucosa with the refluxed content, possibly caused by a defective anti-reflux barrier and luminal clearance mechanisms, would appear to be responsible for the morphological changes in the esophageal epithelium of GERD patients\[^10,20\]\. A well studied ultra-structural alteration, i.e., dilated intercellular spaces (DIS), demonstrated both in ERD and NERD patients\[^21,22\]\. It could explain the genesis of symptoms triggered by the activation of intra-mucosal chemo-sensitive nociceptors and, at the same time, the inflammatory cascade generated by luminal acid diffusing into the tissue. In conditions not associated with severe inflammation, it is unclear how, in the presence of these symptoms, an injurious process initiating in the normal mucosa may lead to macroscopic injuries in a subset of patients (30%-40%), namely the ERD group.

The increased paracellular permeability, associated with the presence of DIS, and the resulting breakdown in the epithelial barrier, do not necessarily result only from excessive acid exposure, as suggested by the normal acid contact time at pH-monitoring in NERD patients, in which the symptoms are generated in the absence of esophageal epithelial erosions\[^23\]\. At the same time, the esophageal mucosa would appear to play a pivotal role in the development of esophageal inflammation and pain. Unlike intestinal inflammation, in which the role of the mucosa has been studied, as far as concerns the esophagus, it has only recently been suggested that gastric juice reflux does not directly damage the esophageal mucosa, but instead stimulates the esophageal epithelial cells to secrete chemokines that attract and activate the immune cells, causing damage to the esophageal squamous epithelial cells\[^23\]\. Microscopic inflammation, characterized by neutrophilic and eosinophilic infiltration of the esophageal mucosa and submucosa, is observed more frequently in ERD than in NERD patients\[^24,26\]\. Therefore, the different esophageal phenotypes of GERD could possibly reflect the presence of various inflammatory mediators responsible for mucosal immune responses in these groups of patients\[^24,27,29\]\. In a recent investigation, we observed that levels of cytokines, such as interleukin-8 (IL-8) and platelet activating factor (PAF), are significantly higher in the esophageal mucosa of ERD patients compared with those in the NERD group, in whom the expression of these inflammatory mediators is comparable to those of controls\[^31\]\. The acid-induced production of IL-8 and other inflammatory mediators by the esophageal mucosa have been shown to promote migration and activation of peripheral blood leukocytes\[^29\]\. These findings would corroborate the hypothesis that a cytokine-mediated mechanism, rather than a direct effect of gastroesophageal reflux, is responsible for the mucosal injury in the ERD subgroup.

Growing evidence shows that NERD patients are characterized by enhanced esophageal sensitivity to chemical and mechanical stimuli caused by enhanced excitability of visceral sensory neurons, possibly associated with overexpression of acid-sensing receptors in the epithelial layer and in the afferent fibers of the lamina propria\[^30,31\]\. In particular, the transient receptor potential channel vanilloid subfamily member-1 (TRPV1) is overexpressed in the esophageal mucosa of ERD and NERD patients,
Table 1 Cytokines and Chemokines involved in the pathophysiology of gastroesophageal reflux disease

| Patients       | Cytokines                   | Chemokines                                      |
|----------------|-----------------------------|-------------------------------------------------|
| GERD           | IL-6, IL-8, and IL-1β       | PAF, MCP-1, RANTES, MIP-1α, Eotaxin-1, Eotaxin-2, |
|                |                             | CINC-2α, and ICAM-1.                            |
| ERD            | IL-6, IL-8, IL-1β, INF-γ,  |                                                 |
|                | TNF-α, IL-1β, IL-8, IL-6   |                                                 |
|                |                             |                                                 |
| NERD           | IL-8, IL-1β                 |                                                 |
| Barrett’s esophagus | IL-8, IL-8α, IL-1β        |                                                 |

GERD: Gastroesophageal reflux disease; ERD: Erosive reflux disease; NERD: Non-erosive reflux disease; IL-6: Interleukin-6; IL-8: Interleukin-8; IL-1β: Interleukin-1β; INF-γ: Interferon-gamma; TNF-α: Tumor necrosis factor-α; PAF: Platelet-activating factor; MCP-1: Monocyte chemoattractant protein; MIP-1α: Macrophage inflammatory protein-1α; CINC-2α: Cytokine-induced neutrophil chemoattractant-2α; ICAM-1: Intercellular Adhesion Molecule-1; RANTES: Regulated upon activation normal T cell expressed and presumably secreted.

INFLAMMATORY MEDIATORS AND GERD PATHOGENESIS

The mucosa of GERD patients produces significantly larger amounts of various cytokines compared with that of healthy controls. These inflammatory mediators activate immune cell recruitment and migration, and may be involved in the pathophysiology of GERD (Table 1).

IL-8, one of the most important neutrophil chemoattractants, is overexpressed in the mucosa of GERD patients, and increased IL-8 levels in the esophageal mucosa of these patients correlate with the endoscopic and histological severity of the disease. Moreover, IL-8 levels decrease following PPIs and following anti-reflux surgery, possibly indicating a role of this chemokine in mucosal damage. Acid-induced production of IL-8 and PAF by the esophageal mucosa promotes the migration of peripheral blood leukocytes. PAF also induces the production of hydrogen peroxide (H₂O₂) by peripheral blood leukocytes.

In a previous study, we have shown that acid-induced inflammation of the esophagus begins with activation of the TRPV1 receptors in the mucosa and synthesis of PAF by the epithelial cells. Furthermore, the sensory neurotransmitters, calcitonin-gene related peptide (CGRP) and substance P, are produced by sensory neurons located in the esophageal mucosal layer.

PAF diffuses from the mucosal layer, stimulating the production of H₂O₂ in leukocytes and the production of IL-6 in the circular muscle, where IL-6 causes production of additional H₂O₂ through activation of NADPH oxidases present in the circular muscle layer. In turn, H₂O₂ triggers the formation of IL-1β, which may induce the production of PAF in the muscle, probably supporting a self-sustaining cycle of inflammatory mediator production. Indeed, in an animal model of chronic esophagitis, significantly increased expressions of other inflammatory mediators, among which IL-1β, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1α) and eotaxins, were detected in esophageal lesions compared with the normal esophagus. In agreement with these previous reports, we recently confirmed, in biopsies from ERD patients, a significant increase in the expression of IL-8, PAF and several chemokines, compared with controls. Interestingly, unlike ERD patients, the esophageal mucosa of NERD patients did not exhibit enhanced expressions of various inflammatory mediators, or the significant presence of neutrophils and eosinophils in the mucosa, being comparable to asymptomatic controls.

This hypothesis is supported by a multicenter, randomized, controlled trial including 514 patients affected by GERD. The study revealed that “microscopic esophagitis” (dilatation of intercellular spaces, papillary elongation and basal cell hyperplasia), was found in more than 90% of GERD patients, but in only approximately 2/3 of NERD patients, with significant infiltration of immune cells only in the ERD group. These findings would indicate a key role of several soluble mediators acting as powerful inflammatory activators, contributing to the induction of esophagitis. The observation that CGRP and substance P are generated by different mechanisms and that two different pathways mediate the sensation of heartburn and inflammation, would further explain the presence of recurrent and severe symptoms, irrespective of mucosal injury.

VISCERAL HYPERSENSITIVITY AND REFLUX PERCEPTIONS IN GERD PATIENTS

The pathogenesis of heartburn and acid regurgitation remain to be fully elucidated, particularly in the numerous NERD patients in whom the 24-h pH-test findings may be within the normal range. Indeed, an enhanced sensitivity to reflux would appear to be strongly associated with the failure of PPI treatment.

Although gastric acid plays a pivotal role in the pathogenesis of GERD, other stimuli have been suggested to be involved in the pathogenesis of typical symptoms. In patients with GERD, reflux may result in direct activation of pain receptors, which may be enhanced by greater acid...
diffusion through the esophageal epithelium caused by impaired mucosal barrier function\[^{[9]}\]. Furthermore, activation of pain receptors may occur following reflux-induced distention of the esophagus. Enhanced esophageal sensitivity to these stimuli may be caused by upregulation of peripheral pain receptors and central sensitization of spinal neurons\[^{[9]}\].

Little is known about acid-sensitive nerves. Receptors on acid-sensitive nerve endings may play a role in nociception and esophageal sensitivity, as suggested in animal models following chronic acid exposure, and include the anion-sensing ion channel (ASIC), TRPV1 and ionotropic purinergic (P2X and P2Y) receptors\[^{[4]}\].

The recently demonstrated presence of acid-sensitive TRPV1 receptors in sensory nerve fibers and in epithelial cells of the esophageal mucosa\[^{[4]}\] provides an interesting mechanism to better understand the onset of neuromodulation and symptoms generation in GERD. TRPV1 activation in primary afferent neurons evokes the sensation of burning pain and may induce neurogenic inflammation following the release of substance P and CGRP\[^{[44]}\].

On the other hand, growing evidence from animal models during chronic acid exposure supports the involvement of purinergic receptors in nociception and hypersensitivity\[^{[19,39]}\]. The purinergic receptors are involved in several cell functions and may be activated by purine nucleotides as ligands\[^{[42]}\].

Based on their pharmacological properties and molecular characteristics, two distinct classes of purinergic receptors with preferential responses to adenosine 5'-triphosphate (ATP), as well as other single nucleotides, have been identified: the family of ligand-gated cation channel P2X receptors and the G protein-coupled P2Y receptors. P2X and P2Y receptors play an important role in signaling visceral pain\[^{[5,9,39,42]}\]. A working hypothesis of purine-mediated mechano-sensory transduction has been suggested\[^{[19,39,41]}\]. ATP released from the epithelial cell lining of the gastrointestinal (GI) tract, bladder and ureter might activate P2X receptors present on the sub-epithelial nerve plexus and the signal is transmitted via the spinal cord to the brain.

To date, a limited number of studies have been performed on changes in purinergic signaling in GI disorders. Extracellular nucleotides and their receptors have been implicated in the pathogenesis of various pathological conditions in the gut; indeed, adenosine increases vagal afferent sensitization in the esophagus and is able to activate a different type of nociceptive nerve terminal in this tissue\[^{[43]}\]. Acid sensitizes P2X receptors to ATP, and acid-induced upregulation and activation of P2X receptors has been confirmed in animal models of esophagitis\[^{[44,46]}\].

However, the role of purinergic receptors in patients with GERD remains to be fully determined. In a recent investigation, we studied a signaling pathway that might be responsible for esophageal nociception, which involves ATP and purinoceptors. In an experimental model of acid-induced activation of the esophageal mucosal nociceptors, we observed that acid exposure caused activation of TRPV1 receptors on the esophageal epithelial cells, triggering production of ATP, which acts on peripheral nerve terminals inducing the production of neurotransmitters\[^{[40]}\]. Thus, the selective presence of purinergic receptors on esophageal epithelial cells was demonstrated, suggesting a direct effect of the acid on the epithelium and a possible autocrine effect of ATP on these cells\[^{[38,39]}\]. In fact, P2X4, P2X5 and P2Y14 receptors are expressed in esophageal epithelial cells, and indeed are expressed at higher levels than all the other purinergic receptors\[^{[46]}\]. P2Y receptors appear to be more involved in esophageal motility. Lecca et al\[^{[41]}\] recently reported that purinoceptors are involved in human lower esophageal sphincter (LES) relaxation, mediated by neural nitric oxide and partially by P2Y receptors. Blockade of P2Y receptors reduced the amplitude of contractions without affecting the latency Farré et al\[^{[44]}\] had previously demonstrated, in animals, that LES relaxation, induced by stimulation of the inhibitory motor neurons of the mesenteric plexus, was mediated by neural nitric oxide with a minor contribution of purines, acting by way of P2Y and P2X receptors.

CONCLUSION

Inflammatory processes in the esophageal mucosa appear to be involved not only in the development of erosive disease, but also in the early and important phases leading to hypersensitivity to intraluminal stimuli.

Despite considerable evidence reconfirming the important production of inflammatory mediators and/or neurotransmitters in the pathogenesis of GERD, the interplay between hypersensitivity and esophageal inflammation remains unclear. Moreover, in the pathogenesis of GERD and in the generation of symptoms, receptors on acid-sensitive nerve endings may play a significant role. Further studies are warranted to better understand the signaling pathway involved in the genesis of reflux symptoms and inflammation, to identify and establish new therapeutic approaches.

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REFERENCES

1. Dent J, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut 2005; 54: 710-717 [PMID: 15831922 DOI: 10.1136/gut.2004.051821]
2. Locke GR, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ. Prevalence and clinical spectrum of gastrooesophageal reflux: a population-based study in Olmsted County, Minnesota. Gastroenterology 1997; 112: 1448-1456 [PMID: 9136821 DOI: 10.1016/S0016-5085(97)0025-8]
3. Camilleri M, Dubois D, Coulie B, Jones M, Kahrilas PJ, Rentz AM, Sonnenberg A, Stanghellini V, Stewart WF, Tack
J. Talley NJ, Whitehead W, Revicki DA. Prevalence and socio-economic impact of upper gastrointestinal disorders in the United States: results of the US Upper Gastrointestinal Study. *Clin Gastroenterol Hepatol* 2005; 3: 543-552 [PMID: 15952060 DOI: 10.1016/S1542-3565(05)0133-9]

Barlow WJ, Orlando RC. The pathogenesis of heartburn in nonerosive reflux disease: a unifying hypothesis. *Gastroenterology* 2005; 128: 771-778 [PMID: 15765412 DOI: 10.1013/j.gastro.2004.08.014]

Kelly M, Hwang JM, Kubes P. Modulating leukocyte recruitment in inflammation. *J Allergy Clin Immunol* 2007; 120: 3-10 [PMID: 17599914 DOI: 10.1016/j.jaci.2007.05.017]

Jiménez P, Piazzuelo E, Sánchez MT, Ortego J, Soteras F, Marianas F. Free radicals and antioxidant systems in reflux esophagitis and Barrett’s esophagus. *World J Gastroenterol* 2005; 11: 2697-2703 [PMID: 15884106 DOI: 10.1016/S0001-5855(03)28656-4]

Oh TY, Lee JS, Ahn BO, Cho H, Kim WB, Kim YB, Surh YJ, Cho SW, Hahn KB. Oxidative damages are critical in pathogenesis of reflux esophagitis: implication of antioxidants in its treatment. *Free Radic Biol Med* 2001; 30: 905-915 [PMID: 11295533 DOI: 10.1016/S0891-5849(01)00472-5]

Fitzgerald RC, Ownebgusi BA, Bajaj-Elliott M, Saeed IT, Cheng L, Ma J, Harnett K, Biancani P, Al-kiekk R, Orlando RC. Dilated intercellular spaces of esophageal mucosa in reflux esophagitis and Barrett’s esophagus. *Gastroenterology* 2009; 137: 1776-1784 [PMID: 19660463 DOI: 10.1016/j.gastro.2009.07.055]

Trimble KC, Pryde A, Heading RC. Lowered oesophageal pH with meal ingestion: a motor correlate of heartburn symptom. *Am J Physiol Gastrointest Liver Physiol* 2004; 287: G791-G798 [PMID: 20616304 DOI: 10.1152/ajpgi.00160.2010]

Souza RF, Hsu X, Mintal V, Schuler CM, Carmack SW, Zhang HY, Zhang Y, Xu C, Hormi-Carver K, Genta RM, Spechler SJ. Acid reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. *Gastroenterology* 2004; 127: 551-556 [PMID: 15133759 DOI: 10.1080/03009035/2004/572539]

Fass R, Ohrnman J. Gastroesophageal reflux disease--should we adopt a new conceptual framework? *Am J Gastroenterol* 2002; 97: 1901-1909 [PMID: 12190152 DOI: 10.1016/S0002-9270(02)04269-7]

Armstrong D. Endoscopic evaluation of gastric-esophageal reflux disease. *Yale J Biol Med* 1999; 72: 93-100 [PMID: 10780570]

Fass R. Erosive esophagitis and nonerosive reflux disease (NERD): combining biologically plausible, pathogenic, antiinflammatory, and therapeutic characteristics. *Clin Gastroenterol Hepatol* 2007; 41: 131-137 [PMID: 17245209 DOI: 10.1017/S0001-503X(07)00702-5]

An evidence-based appraisal of reflux disease management - the Genval Workshop Report. *Gut* 1999; 44 Suppl 2: S1-16 [PMID: 10741335 DOI: 10.1136/gut.44.2008.S1]

Orlando RC. The pathogenesis of gastroesophageal reflux disease: the relationship between epithelial defense, dysmotility, and acid exposure. *Am J Gastroenterol* 1997; 92: 35-55; discussion 55-75: [PMID: 9127620]

Stevenson HE, Riedel R, Heading RC. Lowered oesophageal pH with meal ingestion: a motor correlate of heartburn symptom. *Gastroenterology* 1995; 109: 137-143 [PMID: 7793497 DOI: 10.1016/0016-5085(95)00303-9]

Fass R, Ohrnman J. Gastroesophageal reflux disease--should we adopt a new conceptual framework? *Am J Gastroenterol* 2002; 97: 1901-1909 [PMID: 12190152 DOI: 10.1016/S0002-9270(02)04269-7]

Armstrong D. Endoscopic evaluation of gastric-esophageal reflux disease. *Yale J Biol Med* 1999; 72: 93-100 [PMID: 10780570]

Fass R. Erosive esophagitis and nonerosive reflux disease (NERD): comparison of epidemiologic, physiologic, and therapeutic characteristics. *Clin Gastroenterol Hepatol* 2007; 41: 131-137 [PMID: 17245209 DOI: 10.1017/S0001-503X(07)00702-5]

An evidence-based appraisal of reflux disease management - the Genval Workshop Report. *Gut* 1999; 44 Suppl 2: S1-16 [PMID: 10741335 DOI: 10.1136/gut.44.2008.S1]

Orlando RC. The pathogenesis of gastroesophageal reflux disease: the relationship between epithelial defense, dysmotility, and acid exposure. *Am J Gastroenterol* 1997; 92: 35-55; discussion 55-75: [PMID: 9127620]
32 Rieder F, Cheng L, Harnett KM, Chak A, Cooper GS, Isenberg G, Ray M, Katz JA, Catanzaro A, O’Shea R, Post AB, Wong R, Sivak MV, McCormick T, Phillips M, West GA, Willis JE, Biancani P, Fiocchi C. Gastroesophageal reflux disease-associated esophagitis induces endogenous cytokine production leading to motor abnormalities. *Gastroenterology* 2007; 132: 154-165 [PMID: 17241868 DOI: 10.1053/j.gastro.2006.10.009]

33 Harada A, Sekido N, Akahoshi T, Wada T, Mikuada N, Matsushima K. Essential involvement of interleukin-8 (IL-8) in acute inflammation. *J Leukoc Biol* 1994; 56: 559-564 [PMID: 796163 DOII: 10.1186/ar1917]

34 Isomoto H, Nishi Y, Kanazawa Y, Shikuwa S, Mizuta Y, Inoue K, Kohno S. Immune and Inflammatory Responses in GERD and Lansoprazole. *J Clin Biochem Nutr* 2007; 41: 84-91 [PMID: 18193101 DOII: 10.3164/jcbn.2007012]

35 Oh DS, DeMeester SR, Vallbohmer D, Mori R, Kuramochi H, Hagen JA, Lipham J, Danenberg KD, Danenberg PV, Chandrasoma P, DeMeester TR. Reduction of interleukin 8 gene expression in reflux esophagitis and Barrett’s esophagus with antireflux surgery. *Arch Surg* 2007; 142: 554-559; discussion 559-560 [PMID: 17576892 DOI: 10.1001/archsurg.142.6.554]

36 Ma J, Altomare A, Guarino M, Cicala M, Rieder F, Fiocchi C, Li D, Cao W, Behar J, Biancani P, Harnett KM. HCl-induced and ATP-dependent upregulation of TRPV1 receptor expression and cytokine production by human esophageal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2012; 303: G655-G645 [PMID: 22790593 DOI: 10.1152/ajpgi.00097.2012]

37 Cheng L, Cao W, Fiocchi C, Behar J, Biancani P, Harnett KM. HCl-induced inflammatory mediators in cat esophageal mucosa and inflammatory mediators in esophageal circular muscle in an in vitro model of esophagitis. *Am J Physiol Gastrointest Liver Physiol* 2006; 290: G1307-G1317 [PMID: 16439466 DOI: 10.1152/ajpgi.00386.2008]

38 Hamaguchi M, Fujiiwara Y, Takashima T, Hayakawa T, Sasaki E, Shiba M, Watanabe T, Tominaga K, Oshitani N, Matsumoto T, Higuchi K, Arakawa T. Increased expression of cytokines and adhesion molecules in rat chronic esophagitis. *Digestion* 2003; 68: 189-197 [PMID: 14691346]

39 Knowles CH, Aziz Q. Visceral hypersensitivity in non-erosive reflux disease. *Gut* 2008; 57: 674-683 [PMID: 18079285 DOI: 10.1136/gut.2007.127886]

40 Ribolsi M, Emenenziani S, Pettiti T, Addarri MC, Balestrieri P, Cicala M. Increased frequency and enhanced perception of reflux in non-erosive reflux disease patients non-responders to proton pump inhibitors. *Dig Liver Dis* 2012; 44: 549-554 [PMID: 22366345 DOI: 10.1016/j.dld.2012.01.007]

41 Matthews PJ, Aziz Q, Facer P, Davis JB, Thompson DG, Anand P. Increased capsaicin receptor TRPV1 nerve fibres in the inflamed human oesophagus. *Eur J Gastroenterol Hepatol* 2004; 16: 897-902 [PMID: 15316415 DOI: 10.1097/00042737-200409000-00014]

42 Birder LA. Urothelial signaling. *Autonom Neurosci* 2010; 153: 33-40 [PMID: 19666243 DOI: 10.1016/j.autneu.2009.07.005]

43 Page AJ, O’Donnell TA, Blackshaw LA. P2X purinoceptor-induced sensitization of ferret vagal mechanoreceptors in oesophageal inflammation. *J Physiol* 2000; 523 Pt 2: 403-411 [PMID: 10699084 DOI: 10.1111/j.1469-7793.2000.00403.x]

44 North RA. Molecular physiology of P2X receptors. *Physiol Rev* 2002; 82: 1013-1067 [PMID: 1227951]

45 Bertrand PP. ATP and sensory transduction in the enteric nervous system. *Neuroscientist* 2003; 9: 243-260 [PMID: 12934708 DOI: 10.1177/1073858403255786]

46 Ma J, Altomare A, Rieder F, Behar J, Biancani P, Harnett KM. ATP: a mediator for HCl-induced TRPV1 activation in esophageal mucosa. *Am J Physiol Gastrointest Liver Physiol* 2011; 301: G1075-G1082 [PMID: 21960521 DOI: 0.1152/ajpgi.00336.2011]

47 Lecca B, Gallego D, Farré R, Opazo A, Auli M, Jiménez M, Clavé P. Regional functional specialization and inhibitory nitricergic and nonnitrergic coneurotransmission in the human esophagus. *Am J Physiol Gastrointest Liver Physiol* 2011; 300: G782-G794 [PMID: 21330444 DOI: 10.1152/ajpgi.00514.2009]

48 Farré R, Auli M, Lecea B, Martínez E, Clavé P. Pharmacologic characterization of intrinsic mechanisms controlling tone and relaxation of porcine lower esophageal sphincter. *J Pharmacol Exp Ther* 2006; 316: 1238-1248 [PMID: 16303917 DOI: 10.1124/jpet.105.049482]

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