Clinics in Gastroenterology

ISSN 2690-2494

Review Article

Microbiome and Gastroesophageal Disease: Pathogenesis and Implications for Therapy

Steve M D'Souza[1], Lindsey B Cundra[1], Byung Soo Yoo[1], Parth J Parekh[2] and David A Johnson[3]*

[1]*Department of Internal Medicine, Eastern Virginia Medical School, USA*

[2]*Department of Internal Medicine/Gastroenterology Division, Eastern Virginia Medical School, USA*

[3]*Department of Medicine/Chief of Gastroenterology, Eastern VA Medical School, USA*

Abstract

There is growing evidence that gastroesophageal disease is significantly influenced by the microbiome of the esophagus. Additionally, the commensal microbiome of the oropharynx, stomach, and colon are thought to have a role in the pathogenesis of gastroesophageal disease. Changes to the composition of the normal esophageal flora, notably a transition from Gram-positive to predominantly Gram-negative, affects local microbe-immune cell interaction and is thought to promote disease susceptibility. Diseases of the esophagus, which include Gastroesophageal Reflux Disease (GERD), Barrett's esophagus, esophageal cancer, esophageal dysmotility, as well as eosinophilic esophagitis are well recognized and common with well-established pathogenesis. Emerging data however suggest that these are all characterized by an inflammatory-mediated cascade thought to be propagated, in part, by an imbalance of commensal microbes. In addition to activation of the inflammatory cascade, these dysbiotic changes in bacterial composition may potentiate the process of dysbiosis itself through the expression of bacteriocins. Bacteriocins are inhibitory polypeptides produced by bacteria which may play an integral role in further progression to dysbiotic states by inhibiting growth of commensal flora. While they are mainly used by bacteria to limit proliferation of competitive microbes, isolation of these compounds may present an opportunity for disease mitigation if their inhibitory effects can be targeted to adjust the composition of the microbial community back towards an eubiotic state. Therapeutic options targeting the microbiome, including prebiotics, probiotics, antibiotics, as well as bacteriocins have been studied. This review focuses on the current knowledge of the involvement of the microbiome in esophageal diseases (most notably GERD/Barrett's esophagus/ esophageal cancer) and identifies emerging new concepts for treatment.

Keywords: Microbiome; GERD; Probiotics; Brebiotics; Bacteriocins; Dysbiosis; Barrett's esophagus; Esophageal cancer, Esophagitis

Introduction

Gastroesophageal disease is a major source of health and economic cost worldwide that is estimated at $18.1B annually. Gastroesophageal Reflux Disease (GERD) is the most common esophageal pathology, with 31% of the United States population reporting heartburn or reflux symptoms within the past week [1,2]. Barrett's esophagus (BE), a premalignant complication of GERD, has an estimated prevalence of 5.6% within the United States [3]. Esophageal cancer also poses a significant burden of disease, with >18,000 new diagnoses and >16,000 deaths estimated in 2020 [4]. Changes in understanding of the molecular pathway-driven pathogenesis of the esophageal disease have contributed to the development of many therapeutic options. There has been a recent meteoric rise in the literature demonstrating the significance of the gut microbiome and dysbiosis, defined as microbial imbalance or maladaptation, in the pathogenesis of Gastrointestinal (GI) disease [5]. This review aims to review the current literature for microbiome-related pathogenesis of gastroesophageal disease and to discuss disease-mitigation strategies and future research.

Citation: D'Souza SM, Cundra LB, Yoo BS, Parekh PJ, Johnson DA. Microbiome and Gastroesophageal Disease: Pathogenesis and Implications for Therapy. Clin Gastroenterol. 2020; 2(1): 1008.

Publisher Name: Medtext Publications LLC

Manuscript compiled: Apr 16th, 2020

***Corresponding author:** David A Johnson, Department of Medicine/ Chief of Gastroenterology, Eastern VA Medical School, Norfolk VA, USA, E-mail: dajevms@aol.com

Normal Gastroesophageal Microflora

The esophageal microbiome is shaped by the oral cavity, oropharynx, and stomach due to migration of oral bacteria to the esophagus and reflux of gastric microbiota. There have been multiple attempts to classify the healthy esophageal flora into different cluster types. One study that evaluated healthy and GERD patients demonstrated two microbiome types, referred to as Type I and Type II [6]. Type I microbiomes consisted primarily of Gram-positive microbes, dominated by those within the Streptococcus genus. Type II microbiomes were dominated by Gram-negative flora and were at higher risk of developing reflux-related esophageal disease. One hypothesis for this effect is the activation of Toll-Like Receptors (TLRs) by Gram-negative bacterial products and a subsequent propagation of the inflammatory cascade [7]. Another study further classified esophageal biomes into three community types or clusters: one type dominated by Streptococcus *spp*., one by Prevotella *spp*., and one with intermediate predominance of Streptococcus, Prevotella, *Haemophilus*, and *Rothia spp* [8]. These clusters were associated with a variation in metabolic function. The Streptococcus cluster associated with pentose phosphate metabolism, the Prevotella cluster was associated with Lipopolysaccharide (LPS) production, and, the intermediate cluster was associated with glycolysis and short chain fatty acid (SCFA) production [8]. For all three clusters, progression to reflux-related esophageal disease was associated with increase in relative abundance of Gram-negative flora, which supports the TLR/ inflammatory cascade hypothesis. The composition of esophageal flora is closely linked to the gastric and oral flora. There is a notable similarity of prevalent genera such as Streptococcus, Prevotella, *Haemophilus*, *Fusobacterium*, and *Neisseria*, which are highly

prevalent in both the stomach and oral cavity [9]. One major difference in composition between areas is the presence of Helicobacter pylori in the stomach. This organism, causally linked to gastroduodenal ulceration and inflammation, colonizes the gastric mucosa due to its ability to survive within the strong acidic conditions and is thereby less likely to be found in the esophageal compartment [10,11].

Dysbiosis in Disease States

Gastroesophageal reflux disease

It is well recognized that GERD is an inflammatory disease state affecting the lower esophagus related to transient or chronic lower esophageal sphincter insufficiency. Retrograde reflux of gastric acid with or without bile causes symptoms and inflammatory changes associated with GERD. The most frequent treatment for GERD is directed at gastric acid reduction via acid suppressors such as proton-pump inhibitors (PPIs) or histamine-2 receptor antagonists (H2RAs), antacids as well as lifestyle modification [12]. Untreated GERD may progress further and manifest with complications of erosive esophagitis, esophageal stricture, BE, or esophageal adenocarcinoma [12].

Inflammatory pathogenesis of GERD: The mucosal inflammation seen histologically in GERD was classically thought of as a consequence of direct chemical injury from gastric acid reflux, however, literature has demonstrated an inflammatory-cascade mediated pathogenesis. The specific factors contributing to epithelial insult are mainly gastric acid and duodenal bile salts. There are also many protective factors against the subsequent inflammatory response, such as a barrier of stratified squamous epithelium, paracellular adhesion, and intracellular buffering [13]. Bypassing or overwhelming these protective mechanisms leads to cellular injury and an inflammatory cascade [13]. *In-vitro* and *in-vivo* exposure of lower esophageal keratinocytes to duodenal acidified-bile salts such as those from duodenogastric reflux promotes local cytokine production and migration of lymphocytic cells, primarily T-cells [14]. Progression of exposure leads to inflammation of mucosa but preservation of the epithelial cell layer, implying that the main insult to the mucosa is deep rather than superficial. This suggests that the pathogenesis of reflux esophagitis is primarily inflammation-driven rather than chemically driven. This discounts the intercellular tight junctions and the chemical injury (H+) that ensues after disruption of these tight junctions. A study examining biopsies from patients with GERD before and after treatment with PPIs confirmed proliferation of T-cells, hyperplasia of basal cells, and papillary elongation without damage to surface cells [15]. This study also confirmed the role of PPIs in the reversibility of these histological changes. A key question arises as to the initiating pathogenic event: bile/acid injury direct caustic vs. Gram-negative induced and cytokine related. Non-eroded distal esophageal biopsies in GERD patients show intact epithelial cells as if chemical injury possibly is not the etiology of the inflammation in these areas. We know that through TLR-4/LPS interaction that the cytokine cascade will occur without the chemical injury [16]. Following acid exposure, various inflammatory mediators produced by the mucosa also contribute to lower esophageal sphincter (LES) relaxation. Production of IL (Interleukin)-8, among other induced factors including transient receptor potential channel vanilloid subfamily member-1, substance P, calcitonin gene related peptide, and platelet activating factor, stimulates migration of immune cells. These factors also induce further IL activation and subsequent NADPH oxidase production of hydrogen peroxide. This peroxide effect on local smooth muscle leads to LES relaxation [17]. IL-8 production is also found to be inhibited by PPIs such as omeprazole through a mechanism involving nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activator protein-1, and this may be a contributing factor to the therapeutic effect of PPIs in GERD [18].

Role of the microbiome in GERD: Recognizably, lipopolysaccharide (LPS) is the most abundant and important cell wall constituent of Gram-negative bacteria. It is vital for bacterial cell integrity, viability, and defense against environmental stress [19]. The TLR-4 protein site in humans is the sensing receptor that mediates LPS-induced signal transduction. Following disruption of the epithelial barrier, increased LPS-TLR-4 binding activates production of IL-18, which induces a cascading inflammatory response. This activation of TLR-4 is pivotal for both infectious and noninfectious (e.g. allergic or autoimmune) related inflammation and is a major mechanism for pathogenesis of inflammatory disease states by Gram-negative flora [20]. Further TLR-based signaling promotes transcription of pro-inflammatory chemokines, including IL-1, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) and mediators such as nitric oxide synthase, The chemokines and mediators promote slower esophageal sphincter relaxation and cyclooxygenase (COX)-2 activity, which leads to delayed gastric emptying [21]. This is exemplified in literature discussing *Campylobacter spp*., specifically *Campylobacter concisus*, which is observed only in patients with esophageal pathology [22]. *C. concisus*, a common oral flora, is not often found in the healthy esophagus. However, colonization by the organism was observed at the site of histologic changes due to GERD and BE in addition to a strong correlation between *C. concisus* and an increased level of IL-18 [22,23]. This association suggests the presence of a biofilm, a structurally organized community of flora that stimulate local microbial secretion of an environmentally protective coating and adhesion molecules. Biofilms have been observed in association with GI disease, most notably in oral and colonic pathology [24,25]. Biofilm-associated proliferation may present a framework for understanding esophageal pathology although further research into not just the composition of native flora, but their three-dimensional organization is needed. Therapeutic regimens, especially PPIs, have been demonstrated to alter both the gastroesophageal as well as colonic microbiomes. The use of PPIs in GERD patients has been demonstrated to decrease diversity of gastric, esophageal, and fecal flora [26-28]. It is unclear if this is a protective or injurious effect although this could be a contributing factor for increased infection risk and may play a role in the association with fecal microbiome-related disease such as *Clostridium difficile* infection [29]. The role of *H. pylori* colonization or infection and association with GERD has been controversial. Although patients who are *H. pylori* positive may not demonstrate clinical signs of disease and fall within the "healthy" category, colonization may be associated with reduction of gastric microbial diversity [30,31]. The significance of this effect on esophageal disease is unclear. Several studies have demonstrated an inverse correlation with *H. pylori* infection and severity of reflux-related disease, with support from work suggesting that eradication of *H. pylori* is associated with worsening of GE [32-35]. This effect appears to be limited to specific pro-inflammatory subtypes, including strains associated with gastritis and strains expressing inflammatory cytotoxin-associated gene A (cagA) [36]. It is theorized that decreased acid production in *H. pylori* gastritis-associated strains may contribute to decrease in GERD symptoms. There is some conflicting data as there are several analyses that suggest no association of *H.*

pylori infection with symptoms or pathologic changes associated with GERD nor eradication of infection with increase in GERD symptoms [37-39]. Given the significant morbidity associated with *H. pylori* related peptic ulcer disease, eradication remains the current recommendation. Further research into the inflammatory mechanism behind the protective effects of specific subtypes, however, may play a role in future therapeutic approaches to GERD. It has been theorized that metabolic activity of the colonic biome may further contribute to GERD progression [40]. Colonic breakdown of fermentable oligo-, di-, monosaccharides and polyols (FODMAPs) produces short-chain fatty acids (SCFAs) that contribute to lower esophageal sphincter (LES) relaxation [41,42]. The mechanism for this may be through stimulation of Peptide YY (PYY) production [43]. Both SCFA as well as PYY act to inhibit gastric motility as well as to relax lower esophageal sphincter tone, resulting in retention of gastric contents as well as susceptibility to reflux, which has implications for dietary intake and colonic microbiome alteration within the scope of GERD [42].

Barrett's esophagus

Barrett's esophagus is an intestinal metaplasia of the distal esophageal epithelium characterized by transition from normal stratified squamous epithelial composition to columnar mucosa [44,45]. This occurs in response to chronic inflammation of mucosa secondary to reflux, and it carries a risk for esophageal adenocarcinoma (EAC) [6,21,46].

Inflammatory pathogenesis of BE: As seen in the pathogenesis of GERD, expression of proinflammatory cytokines such as IL-1B, IL-6, and IL-8, has a close causational relationship. These cytokines are also linked to the transition to metaplasia seen in BE. A mouse model of BE demonstrated that IL-1B and IL-6 are overexpressed at the squamocolumnar junction of the esophagus and promote an inflammation of gastric cardiac stem cells [47]. The inflammation further leads to alteration of stem cell niche and provides a pathway for progression to dysplasia. Another mouse model of Barrett's esophagus has revealed that a high fat diet (HFD) is associated with esophageal dysplasia through alteration of the microbiome [48]. HFD increases production of IL-8/C-X-C Motif Chemokine Receptor 1 chemokines, known to be upregulated in a pro-inflammatory state, and the cytokines stimulate the migration of immature granulocytic cells in the esophagus promoting local inflammatory responses. With the predominance of Gram-negative species in the distal esophagus via the increased LPS ligands, as seen in GERD, there is a consequent pro-inflammatory response through an increased release of chemokine/cytokine. The host's response to the increased Gram-negative LPS results in NF-κB activation of the epithelial cells. NF-κB molecular pathway serves as an initial responding step from noxious stimuli (chemical, bacterial, and viral) and assumes a role of upregulating inflammation, innate immune responses, adaptive responses, apoptosis inhibition, cell proliferation and differentiation. IL-1B and IL-8 are increased as a result of NF-κB activation and the secreted cytokines create a positive feedback loop eliciting a more robust innate immune response in BE.

Role of the microbiome in BE: As seen in patients with GERD, patients with BE have a distinct microbiome composition [49]. Type II microbiomes, as described earlier, are associated with progression of GERD to BE [50]. The species in dominance are *Veillonella,* Prevotella, *Haemophilus, Neisseria, Fusobacterium, Rothia, Granulicatella, Campylobacter, Porphyromonas, Fusobacterium*, and *Actinomyces spp*. Type II microbiomes *demonstrate* a decrease in Streptococcus spp. and an increase in Gram-negative anaerobes/microaerophiles including *Veillonella*, Prevotella, *Haemophilus*, *Neisseria*, *Rothia*, *Granulicatella*, *Campylobacter*, *Porphyromonas*, *Fusobacterium*, and *Actinomyces spp*. This transition from Gram-positive to Gram-negative predominance is what is believed to be a contributing factor to pathogenesis of esophagitis as well to drive metaplastic progression to BE [50,51]. Expression of LPS from Gram-negative bacteria, and subsequent activation of the TLR-4-NF-κB pathway is associated with expression of IL-8 and COX-2, and levels of both are directly correlated with transition from metaplasia to dysplasia [52]. It is also possible that gastric acid could contribute to conversion from Type I to Type II microbiome by killing acid-sensitive bacteria in the esophagus [53]. Molecular products secreted by these flora or components of the bacterial wall such as LPS interact with TLRs and continue the inflammatory cascade seen in reflux esophagitis, preventing resolution of mucosal changes. During the ongoing inflammatory process of reflux esophagitis, changes in the local flora predispose the local squamous epithelial tissue towards metaplasia to columnar epithelium. Wild-type murine models given a HFD demonstrated increase in goblet cell prevalence and relative neutrophil presence compared to germ-free models. This suggests that local microbe-epithelium interactions, presumed to be through microbe product-TLR binding, is a possible mechanism for the metaplastic process. Furthermore, there may be a role of the colonic microbiome in this process as an increase of the *Firmicutes:Bacteroidetes* ratio within the colon is seen in BE. *Firmicutes spp*. metabolize FODMAPs and dietary fiber into SCFAs. As previously described, SCFA production is associated with decreased LES tone as well as decreased gastric motility through stimulation of PYY mediated smooth muscle effects. There is considerable overlap between periodontal and esophageal flora, with a similar high *Firmicutes/Bacteroidetes* ratio [54]. It is presumed that this similarity in composition is mainly from movement of microbes in an aboral direction. However, a recent study found a distinct oral microbiome that is associated with presence of BE.49 This case-control study analyzed the oral microbiome using a three-taxon model, *Lautropia*, Streptococcus, and unspecified genus in the order of *Bacteroidales* and distinguished the microflora of patients with BE from healthy individuals. This model of clustering predicted patients with BE with a sensitivity of 97% and specificity of 88%. The model suggests decreased abundance of *Lautropia* and increased abundance of Streptococcus and *Enterobacteriaceae* in BE patients. The increased presence of *Lautropia* and *Enterobacteriaceae*, both Gram-negative, correlates with the LPS/TLR-4 hypothesis, but increased Streptococcus abundance suggests that pure Gram-positive/negative ratio may provide a complete explanation. More broadly, increased *Firmicutes spp*. suggests that SCFA production may also play a role in BE pathogenesis. Larger investigations are needed to further evaluate the role of oral dysbiosis and diagnostic implications for BE as various environmental factors including diet can influence the oral microbiome and larger sample size is needed to isolate the predictive effect [55].

Esophageal Cancer

Esophageal cancer is one of the most frequent cancers worldwide. Squamous cell carcinoma and adenocarcinoma comprise the two major histological subtypes.56 Globally, esophageal squamous cell carcinoma (ESCC) is the more common of the two, making up approximately 88% of esophageal cancers [56,57]. Risk factors for both malignancies overlap, their etiologies and incidence vary [58].

Inflammatory pathogenesis

Adenocarcinoma: The etiology of EAC has been associated with long standing inflammation or mucosal injury, such as that seen in reflux esophagitis. The pathophysiology contributing to this disease state is complex involving an interplay between environmental factors, genetic susceptibilities, and host dynamics. However, approximately 80% of cases can be attributed to GERD, cigarette smoking, obesity, and low fruit and vegetable consumption [59]. GERD is the strongest risk factor for EAC, correlating strongly with duration and frequency of symptoms. Other risk factors include BE, motor disorders of the esophagus, other malignancies, medications, and environmental exposures [60]. BE is the risk factor that has the strongest association with EAC with estimates that BE progresses to high-grade dysplasia at a rate of 0.5% to 0.9% per year [61]. However, EAC, like the complications of GERD discussed above, can arise without preceding symptoms. As discussed previously, the pathogenesis of GERD follows an inflammatory-mediated cascade rather than through direct mucosal injury. On the contrary, GERD is not a risk factor for ESCC, which is additionally associated with age, socioeconomic status, alcohol consumption, and human papillomavirus. Recent epidemiological studies have observed that the incidence of EAC to be on the rise in the Western world, with a 6-fold increase in the United States alone [62]. The recent rise in incidence in the Western world suggests an environmental etiology at play. Studies have explored factors such as diet, smoking, obesity, *H. pylori* infection, and antibiotics.

Squamous cell carcinoma: Esophageal squamous cell cancer is a complex disease, with many predisposing factors, involving both genetic as well as environmental components. Incidence of the disease is influenced by environmental exposure, but there is regional variation to the nature of exposure. The highest areas of incidence include East Asia, Southeastern Africa, and Southeastern South America. Smoking and tobacco use are the most common risk factors in wealthier populations, while hot-liquid consumption, dietary carcinogens, and poor dentition contribute enough to incidence that poverty itself is a risk factor for pathogenesis [57,63,64]. There is a growing body of literature evaluating genetic susceptibility that may increase carcinogenesis following an environmental trigger. Heavy alcohol consumption is a strong risk factor for ESCC. Acetaldehyde, a direct metabolite of ethanol oxidation, inhibits DNA repair through a variety of mechanisms. Ethanol may also directly induce production of reactive oxidation species and promotes aberrant epigenetic modification, in particular DNA methylation [65]. Abnormal methylation of genes associated with carcinogenesis inhibits expression of tumor-suppressor genes and promotes oncogene transcription and is the major proposed mechanism for direct carcinogenic effect of ethanol [66]. Pathogenesis of ESCC is associated with overexpression of inflammatory mediators. Persistent production of NF-κB and activation of TLR-4 have both been demonstrated to be present in early stage ESCC and are decreasing with progression to advanced stages [67]. TLR-4 in particular activates an innate inflammatory response with subsequent activation of an acute to chronic inflammatory cascade [68]. This suggests that the presence of external factors that affect the local mucosa-microbe interaction leads to a localized inflammatory reaction, and that persistence of this inflammation coupled with a genetic predisposition triggers hyperproliferation of squamous tissue and progression to carcinoma.

Role of the microbiome in esophageal cancer

There is a growing body of evidence investigating the relationship between the microbiome and esophageal cancer [69]. The microbiome is altered in precursors to esophageal carcinoma, such as the abnormal Type II microbiomes with enriched gram-negative bacteria that are mainly associated with GERD and BE. This alteration of the microbiome is potentially involved toward carcinogenesis. The microbiota of cancerous esophageal tissue has been characterized to be profoundly affected by the oral microbiome and periodontopathic species derived from the oral cavity. Oral microbial composition has been associated with risk of EAC and ESCC [69]. In fact, a multitude of studies implicate oral bacteria in the etiology of oral, esophageal, gastric and other gastrointestinal cancers [70]. Due to bacteria migration, the oral and gastric microbiota shape the esophageal microbiome and therefore may contribute to esophageal carcinogenesis. However, there are variations in microbiota even between ESCC and EAC.

Adenocarcinoma: The microbiome of EAC has been characterized predominantly by periodontopathic species derived from the oral microbiome: *Treponema denticola,* Streptococcus *mitis*, and Streptococcus *anginosus* [71]. The latter two are Gram-positive and this suggests a different pathway (i.e. migration or SCFA metabolism, as described in the BE pathogenesis pathway) than the previously discussed LPS/dysbiosis pathway. This suggests that periodontitis and inadequate oral hygiene may be associated with increased esophageal cancer risk [72]. In particular, fragments of *S. anginosus* has been isolated in head and neck carcinomas as well as in early dysplastic changes of esophageal and gastric cancer [73]. This implies that *S. anginosus* is associated with numerous malignancies of the upper digestive tract. The exact mechanism underlying this process has not been delineated. However, induction of inflammatory cytokines by infection of *S. mitis* and *S. anginosus* has been demonstrated. Other periodontal species have been associated with esophageal cancer. For instance, it has been found that the periodontal pathogens *Tannerella forsythia, Veillonella, Selenomonas*, and *Treponema denticola* to be associated with higher risk of EAC [74,75]. On the contrary, decreased Streptococcus prevalence is associated with an increased risk of EAC. This implies that specific flora are inversely related to malignant transformation. Further evidence of this inverse correlation is provided by lower EAC risk is associated with infection with *H. pylori*. In one study, the microbiome in both normal subjects and EAC was characterized to be more alike than to BE comparisons, with an increased relative abundance of *Bifidobacteria, Bacteroides, Fusobacteria, Veillonella, Staphylococcus* and *Lactobacilli* and decreased relative abundance of Campylobacter when compared with BE samples. Other protective factors such as bacterial biosynthesis of carotenoids by oral *Neisseria spp*. were also associated with protection against EAC. This suggests that commensal colonization by specific flora may be protective from the metaplastic process by inhibiting proliferation of pro-inflammatory flora as well as through synthesis of vitamins. However, increased EAC risk has also been associated with depletion of certain bacteria. For instance, depletion of the commensal genus Neisseria and the species Streptococcus pneumoniae are associated with higher EAC risk. This is corroborated by other studies wherein microbial diversity has also been shown to be decreased [75,76]. It has been postulated that once carcinogenesis has begun, Streptococcus leave the local environment to invade surrounding tissue [77]. Further, the microbiota associated with EAC may change depending on other risk factors such as obesity, complicating the findings. Toll-like receptors (TLRs) are

a class of proteins that play a key regulatory role within the innate immune system. A potential mechanism by which the microbiome participates in carcinogenesis, is via TLRs.75 TLRs 1-3, 6, 7 and 9 are significantly upregulated in EAC [77]. Both TLR-4 and TLR-5 have also been suggested as potential mediators of the progression from reflux disorders to EAC. This suggests an association between the TLR signaling pathway and the altered microbiome. In tissue biopsies from the esophagus, TLR-4 (whose natural ligand is LPS) expression is significantly increased in EAC and BE when compared to normal esophagus. Further, activation of the TLR-4-NF-κB pathway is evident in reflux disorders and may contribute to malignant transformation. Therefore, in the abnormal Type II microbiomes, where there is a predominance of Gram-negative bacteria, overstimulation of TLR-4 may trigger a larger and more carcinogenic inflammatory cascade. Further, expression of the COX-2 isoform, an LPS-TLR-4-NF-κB pathway downstream gene, is elevated in esophageal carcinomas. It has been found that there is an increase of COX-2 that occurs along the progression from low-grade dysplasia to high-grade dysplasia in the EAC pathway [78]. This implies that the activation of the LPS-TLR-4-NF-κB pathway may contribute to malignant transformation. This theory has been experimentally tested in a murine model wherein the presence of *E. coli* induced activation of TLRs implicated in EAC.

Squamous cell carcinoma: As with esophageal adenocarcinoma, ESCC has been shown to be associated with periodontal pathogens and poor oral hygiene. Specifically, the abundance of the periodontal pathogen *Porphyromonas gingivalis* trended with higher risk of ESCC [79]. In one study, poor oral health was reported as a risk factor for esophageal squamous dysplasia [80]. To this point, oral SCC, also associated with poor oral hygiene, has been linked to changes in the oral microbiome (*Firmicutes,* Streptococcus*, Actinobacteria*, and *Rothia*), which were substantially decreased in relation to normal tissue [81]. Oral SCC has been shown to be accompanied with other squamous cell carcinomas of the digestive tract [82]. It has been suggested that a region of epithelial cells can be affected by carcinogenic alterations [83]. Subjects with ESCC have also been shown to exhibit decreased microbial diversity. Interestingly, this decrease in microbial diversity has been replicated in other anatomical sites of the gastrointestinal system such as the stomach with gastritis and the colon with colorectal cancer [84]. Gastric microbiota changes have also been associated with ESCC, and *Clostridiales* and *Erysipelotrichales* orders have been particularly implicated [85]. In addition to direct carcinogenic effect, environmental factors such as alcohol and tobacco may alter the local microbiome and contribute to carcinogenesis indirectly. While is no literature characterizing the esophageal flora in patients with heavy alcohol or tobacco use, dysbiosis in the oral cavity and colon may contribute through similar mechanisms. Consumption of alcohol in patients with oral microflora abundant with oxidizing flora leads to production of acetaldehyde, and subsequent DNA repair inhibition, leading to increased susceptibility to oral squamous cell carcinoma [86,87]. Tobacco use is associated with increased abundance of Streptococcus spp. and yeast capable to metabolizing alcohol to acetaldehyde, as well as inhibiting acetaldehyde breakdown, suggesting that persistence of salivary aldehyde can contribute to esophageal carcinogenesis [88]. Alcohol use is associated with decrease in murine fecal *Firmicutes* and *Bacteroidetes*, but a more pronounced decrease in *Bacteroidetes* results in an increased *Firmicutes:Bacteroidetes* ratio [89]. This increased ratio alters local nutrient metabolism and increases serum LPS levels, and may suggest another mechanism for pathogenesis, though a clear linkage has not been established. Alcohol may also influence both local and systemic response to microbe-immune crosstalk. Locally, ethanol inhibits epithelial cell expression of tight junction-associated proteins, zonula occludens-1 and claudin-1, increasing barrier permeability and susceptibility to the LPS-mediated inflammatory response [90]. Systemically, both heavy chronic and acute ethanol consumption may also decrease clearance of LPS from the bloodstream, potentiating a systemic pro-inflammatory effect [91].

Esophageal Dysmotility

Inflammatory pathogenesis

Altered neuromotor function leading to gastrointestinal dysmotility in response to mucosal inflammation has been described in esophagitis and ulcerative colitis [92]. In patients with GERD, an increase in cytokines and chemokines such as IL-1B, IL-6, IL-8, IL-10, Interferon-γ, monocyte chemoattractant protein-1, and Regulated-upon-Activation, Normal-T-Cell-Expressed-and-Presumably-Secreted (RANTES) are observed [93]. IL-6, a cytokine released as a result of mucosal damage, affects the circular smooth muscle cells in the lower third of the esophagus and ultimately disturbs muscle contractility. Although the exact mechanism of cytokine effects on neuroafferent cells is unknown, it is proposed that the increased number of cytokines are produced in exposure to gastric refluxate and/or exposure to dysbiosis/LPS in GERD. These cytokines, including IL-6, are able to alter the esophageal contractility leading to esophageal dysmotility [94].

Role of the microbiome in esophageal dysmotility

While there are no studies directly evaluating the role of the local microbiome in pathogenesis of esophageal motility disorders, there are some investigations that have characterized the microbiome in achalasia, Chagas disease as well as connective tissue diseases such as systemic sclerosis. Additionally, a large volume of literature characterizes the effects of local flora on smooth muscle elsewhere along the GI tract, particularly the colon. Although Chagas disease is a well-recognized consequence of infection by a tropical parasitic disease caused by *Trypanosoma cruzi*, samples of flora grown from patients with Chagas related megaesophagus has demonstrated a predominance of nitrite- and nitrate-reducing bacteria [95]. Further investigation of patients with achalasia and megaesophagus demonstrate an overgrowth of Streptococcus *spp*., many of which may act as nitrite-/nitrate-fermenters [96]. As previously described, Type I esophageal microbiomes are typically seen as 'normal' flora and consist primarily of Streptococcus *spp*. It is possible that the decrease in interaction between esophageal and gastric flora in various megaesophagus states leads to overgrowth of these Type I flora, although this has not clearly been characterized. Furthermore, increase in nitrite-/nitrate- fermentation, coupled with increased esophageal retention of food products may contribute to development of squamous cell carcinoma and may explain the increased risk that is seen in these patients. As described previously in relation to GERD and BE, increase in the fecal *Firmicutes/Bacteroidetes* ratio corresponds with an increase in *Firmicutes spp*. Fermentation products, specifically SCFAs. SCFA and associated PYY downstream effects on gastric smooth muscle lead to decreased contraction. This SCFA mediated effect following change in colon *Firmicutes:Bacteroidetes* ratio has also been demonstrated in cases of decreased colonic motility leading to constipation [97]. While their effect in the terminal ileum appears to be mainly stimulation of peristalsis following ileocolic reflux, effects elsewhere in the GI tract mainly appear to be decrease in motor

tone [98]. More investigation is needed to isolate the effect of SCFA production on esophageal peristalsis. Connective tissue diseases are associated with gut dysbiosis [99]. Most notably, systemic sclerosis, which can lead to fibrosis of the muscular layers of the GI tract and subsequent dysmotility, most frequently manifests in the esophagus [100]. Multiple studies investigating compositional changes in colonic flora have demonstrated decrease in commensal flora such as *Bacteroides*, *Clostridium*, and *Faecalibacterium*, and increase in invasive flora [101-104]. While the mechanism for dysbiotic contribution to fibrosis still requires more investigation, it is believed that epithelial barrier dysfunction leading to microbial inflammatory cascade activation may play a role [104].

Eosinophilic Esophagitis

Eosinophilic Esophagitis (EoE) is a chronic immune mediated disorder that is characterized endoscopically by fixed esophageal rings, esophageal narrowing, and mucosal friability and diagnosed by biopsy findings of eosinophilic infiltration of the esophageal mucosa [105]. It is frequently associated with atopic disease, most frequently in male patients, and initially treated with a combination of an anti-inflammatory regimen, most often topical corticosteroids, and dietary modification to remove potential allergens [105].

Inflammatory pathogenesis

EoE is a multifactorial disease, with several genetic components, frequently atopic condition associations, as well as environmental factors that are presumed to affect pathogenesis. The role of these various factors in development of EoE is still being characterized, and there are several explanations for its inflammatory origins. One theory is that repeat allergen exposure in susceptible individuals may contribute to eosinophil-driven inflammation [106]. This is highlighted in some data that demonstrates childhood PPI use with EoE as theoretically decreasing allergen digestion and prolonging exposure [107]. The progression of EoE is believed to be following genetic or environmental disruption of the epithelial barrier exposing the underlying mucosal tissue to local allergens and bacterial products. These products stimulate secretion of IL-1, IL-8, and migration of T-helper 2 (Th2) cells, which produce IL-5 and stimulate recruitment and activation of eosinophils, as well as IL-13, which stimulates downregulation of desmoglein-1, a cell adhesion molecule integral to the maintenance of the epithelial barrier [108,109]. Local activation of eosinophils leads to toxic degranulation that further simulated local inflammation as well as transforming-growth factor (TGF) expression, which mediates structural protein deposition and leads to the characteristic endoscopic and histologic fibrotic findings [109,110].

Role of the microbiome in EoE

Esophageal mucosal eosinophilia and its associated diseases are characterized by a change in the local microbiome [111]. It has been reported that in patients with active EoE, the microbiome contains a significant increase in *Haemophilus spp*. Furthermore, this change was mitigated following standard of care EoE treatment to a microbiome found in GERD and healthy subjects. Notably, the bacterial load but not the diversity was increased in subjects with EOE and GERD as compared to healthy controls. In another study however, patients with active EoE had a significant increase in *Neisseria spp*. and *Corynebacterium spp*. compared with controls with mitigation of effect following standard of care EoE treatment [112]. *Haemophilus* and *Neisseria* are both genera within the *Proteobacteria* phylum, and are associated with activation of the inflammatory cascade [113]. Composition of more proximal flora may also contribute to pathogenesis of EoE. A study of the salivary microbiome in pediatric patients demonstrated significant differences in bacterial composition in EoE patients compared to non-EoE controls [114]. Most specifically, there was an association with *Haemophilus spp*. with active disease, similar to the changes seen in esophageal samples. This suggests a close interaction between the flora of both locations, and that characterization of salivary dysbiosis may be a surrogate marker for EoE disease activity.

Role of Bacteriocins

Bacteriocins are small peptide molecules that are expressed by bacteria in response to stress. Their primary role is presumed to be inhibition of competitive local flora, but they play a larger role in regulation of the microbiota within the human GI tract. They have been traditionally used with the food industry internationally for preservation, but their role in combination with antibiotics as well as their role in inhibition of carcinogenesis are still developing [115]. These bacterial products are typically classified by nature of bactericidal activity, genetic structure, size, and method of production, and they can separately be classified by expression from Gram-positive or Gram-negative flora [116]. A clear direct effect of bacteriocins would include direct antibiotic effect or incorporation as adjuvants into the current antibiotic generation. These inhibitory effects lead to changes in composition of the local flora. The local stress on the commensal flora may induce Gram-negative bacteriocin production and drive the local flora composition to be more Gram-negative dominant, or towards a Type II microbiome. This may contribute to the esophageal dysbiosis that is associated with progression of reflux-related disease. Another potential role of bacteriocins is in their antineoplastic effect. Thus far, the majority of research regarding their role has been on the colorectal adenoma-adenocarcinoma pathway. In this pathway, increase in bacteriocins from Gram-negative bacteria, specifically microcins and colicins, has been documented to correlate with progression of disease. This increased bacteriocin concentration consists primarily of specific expression of Gram-negative derived colicins and microcins. These small polypeptides are both protective against competitive flora as well as promote direct cytotoxic effect on local tumor cells, though this effect is still being clarified. In the EAC pathway, it is possible that a similar shift in bacteriocin production may contribute to local cytotoxic effect, though a specific cytokine mediated pathway is still being identified.

Implications for therapy

Prebiotics

There is increasing evidence that dietary intake has a profound effect on microbiome balance. This effect plays a key role in reducing dysbiosis related induction of inflammatory signaling cascades [117-119]. Due to the developing understanding of the role of the microbiota, especially Gram-positive/Gram-negative ratio, in the pathogenesis of gastroesophageal disease, this presents a promising avenue for therapeutic intervention through utilization of bacteriocin based therapies. Bacteriocins, as mentioned in earlier sections, are bacterial polypeptide products that target a narrow spectrum of competitive flora and inhibit growth. They are also inactivated by enzymatic degradation, decreasing toxicity and limiting diffuse inhibitory effects. Prebiotics, including Maltosyl-Isomaltooligosaccharide (MIMO), are aimed at improving Gram-positive/Gram-negative ratio. Isomaltooligosaccharides have been demonstrated at increasing

the number of Gram-positive flora, especially *Lactobacillus spp*. It is theorized that this increase in Gram positives coincides with an increase in bacteriocins that inhibit proliferation of Gram-negative flora. This intervention type has been evaluated with some potential in reducing or eliminating GERD symptoms in case series [120].

Probiotics

The use of probiotics to modify the gut microbiome has been trialed in a variety of GI disease states, and there are several investigations into usage to decrease GERD symptoms. Probiotic formulations that include strains mainly within the genera *Lactobacillus* and *Bifidobacterium* have been studied and have demonstrated reduction in symptoms when used as monotherapy [121-124]. However, many studies are limited by quality due to limitations in experimental design such as producing an adequate placebo for control [125]. A randomized-controlled study utilizing *Bacillus subtilis and Enterococcus faecium with PPI* demonstrated decrease in diarrhea symptoms and small intestinal bacterial overgrowth but did not reduce GERD symptoms or healing rate compared to PPI alone [126]. Additionally, recent evidence demonstrated that although orally administered probiotics can remain viable, there may be a marked mucosal colonization resistance by the host [127].

Antibiotics

Antibiotics are also a possible therapeutic option for modifying the microbiome, given their efficacy in the treatment of GI infectious diseases. While routine use of antibiotics for the treatment of esophageal disease has not been investigated, it has been used with success elsewhere in the GI tract for dysbiosis related disease states. In patients with Small Intestinal Bacterial Overgrowth (SIBO), a condition characterized by proliferation of commensal flora within the small bowel, antibiotics with poor oral bioavailability such as rifaximin have been used with efficacy, though data on the use of systemic antibiotics is limited [128]. Given the implications of emerging bacterial resistant pathogens and risk for *C. difficile colitis*, it is not likely that this approach will be viable for esophageal diseases [129,130].

Bacteriocins

Another possible avenue for intervention is direct bacteriocin utilization and delivery. As previously mentioned, bacteriocins have a potential use through two mechanisms, direct antibiotic effect as well as cytotoxic effect towards neoplastic cells. While this is an active area of research, and inhibitory isolates that target Methicillin-resistant *Staphylococcus aureus* [131] and Vancomycin-resistant Enterococcus [132] have been isolated, more research is needed regarding products that target primarily Gram-negatives as well as subsequent literature to evaluate for efficacy and safety [132]. Two potential weaknesses that bacteriocins as therapeutic agents may have is decreased efficacy due to early digestion of products prior to reaching the target site as well as cytotoxic effects on non-target tissues. Fortunately, one promising solution is utilization of similar methods for target drug delivery, encapsulation or attachment to bacteriocins to macromolecule-based, metal, or polymer-based nanoparticles [133]. This has the added potential for modulating or increasing intended effect through attachment of adjuvants to the same nanodelivery particles.

Conclusions

Dysbiosis of the local flora within the esophagus is associated with progression to various gastroesophageal disease states, including the gastroesophageal reflux disease, reflux esophagitis, Barrett's esophagus, esophageal adenocarcinoma, esophageal squamous cell carcinoma, dysmotility, and eosinophilic esophagitis. Dysbiosis manifests through various mechanisms, including upregulation of inflammatory pathways through altered gut-microbe interaction as well as changes in composition through secretion of bacteriocins and subsequent local bactericidal and cytotoxic effects. Further understanding of these interactions may suggest potential therapeutic options. At present, prebiotics and perhaps direct bacteriocin therapies have the most promising potential for esophageal disease.

References

1. Almario CV, Ballal ML, Chey WD, Nordstrom C, Khanna D, Spiegel BMR. Burden of Gastrointestinal Symptoms in the United States: Results of a Nationally Representative Survey of Over 71,000 Americans. Am J Gastroenterol. 2018;113(11):1701-10.
2. Peery AF, Crockett SD, Murphy CC. Burden and Cost of Gastrointestinal, Liver, and Pancreatic Diseases in the United States: Update 2018. Gastroenterology. 2019;156(1):254-72.
3. Hayeck TJ, Kong CY, Spechler SJ, Gazelle GS, Hur C. The prevalence of Barrett's esophagus in the US: estimates from a simulation model confirmed by SEER data. Dis esophagus Off J Int Soc Dis Esophagus. 2010;23(6):451-7.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30.
5. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Heal Dis. 2015;26(0).
6. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and Intestinal Metaplasia of the Distal Esophagus Are Associated With Alterations in the Microbiome. Gastroenterology. 2009;137(2):588-97.
7. Ajayi TA, Cantrell S, Spann A, Garman KS. Barrett's esophagus and esophageal cancer: Links to microbes and the microbiome. PLoS Pathog. 2018;14(12):e1007384.
8. Deshpande NP, Riordan SM, Castaño-Rodríguez N, Wilkins MR, Kaakoush NO. Signatures within the esophageal microbiome are associated with host genetics, age, and disease. Microbiome. 2018;6(1).
9. Bassis CM, Erb-Downward JR, Dickson RP. Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals. MBio. 2015;6(2).
10. Marshall BJ, Warren JR. Unidentified Curved Bacilli in the Stomach of Patients with Gastritis and Peptic Ulceration. Lancet. 1984;323(8390):1311-5.
11. Scott DR, Marcus EA, Weeks DL, Sachs G. Mechanisms of acid resistance due to the urease system of Helicobacter pylori. Gastroenterology. 2002;123(1):187-95.
12. Katz PO, Gerson LB, Vela MF. Guidelines for the diagnosis and management of gastroesophageal reflux disease. Am J Gastroenterol. 2013;108(3):308-28.
13. Orlando RC. The integrity of the esophageal mucosa. Balance between offensive and defensive mechanisms. Best Pract Res Clin Gastroenterol. 2010;24(6):873-82.
14. Souza RF, Huo X, Mittal V. Gastroesophageal Reflux Might Cause Esophagitis Through a Cytokine-Mediated Mechanism Rather Than Caustic Acid Injury. Gastroenterology. 2009;137(5):1776-84.
15. Dunbar KB, Agoston AT, Odze RD. Association of acute gastroesophageal reflux disease with esophageal histologic changes. JAMA - J Am Med Assoc. 2016;315(19):2104-12.
16. Mazgaeen L, Gurung P. Recent Advances in Lipopolysaccharide Recognition Systems. Int J Mol Sci. 2020;21(2):379.
17. Harnett KM, Rieder F, Behar J, Biancani P. Viewpoints on Acid-Induced Inflammatory Mediators in Esophageal Mucosa. J Neurogastroenterol Motil. 2010;16(4):374-88.
18. Huo X, Zhang X, Yu C. In oesophageal squamous cells exposed to acidic bile salt medium, omeprazole inhibits IL-8 expression through effects on nuclear factor-κB and activator protein-1. Gut. 2014;63(7):1042-52.
19. Sweet MJ, Hume DA. Endotoxin signal transduction in macrophages. J Leukoc Biol.

1996;60(1):8-26.

20. Liaunardy-Jopeace A, Gay NJ. Molecular and cellular regulation of Toll-like receptor-4 activity induced by lipopolysaccharide ligands. Front Immunol. 2014;5(OCT).

21. Yang L, Francois F, Pei Z. Molecular pathways: Pathogenesis and clinical implications of microbiome alteration in esophagitis and barrett esophagus. Clin Cancer Res. 2012;18(8):2138-44.

22. Blackett KL, Siddhi SS, Cleary S. Oesophageal bacterial biofilm changes in gastro-oesophageal reflux disease, Barrett's and oesophageal carcinoma: Association or causality? Aliment Pharmacol Ther. 2013;37(11):1084-92.

23. Corning B, Copland AP, Frye JW. The Esophageal Microbiome in Health and Disease. Curr Gastroenterol Rep. 2018;20(8).

24. Marsh PD. Dental plaque as a biofilm and a microbial community - implications for health and disease. BMC Oral Health. 2006;61):S14.

25. Dejea CM, Fathi P, Craig JM. Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. Science. 2018;359(6375):592-7.

26. Imhann F, Bonder MJ, Vila AV. Proton pump inhibitors affect the gut microbiome. Gut. 2016;65(5):740-8.

27. Jackson MA, Goodrich JK, Maxan ME. Proton pump inhibitors alter the composition of the gut microbiota. Gut. 2016;65(5):749-56.

28. Macke L, Schulz C, Koletzko L, Malfertheiner P. Systematic review: the effects of proton pump inhibitors on the microbiome of the digestive tract—evidence from next-generation sequencing studies. Aliment Pharmacol Ther. 2020;51(5):505-26.

29. Janarthanan S, Ditah I, Adler DG, Ehrinpreis MN. Clostridium difficile-associated diarrhea and proton pump inhibitor therapy: A meta-analysis. Am J Gastroenterol. 2012;107(7):1001-10.

30. Llorca L, Pérez-Pérez G, Urruzuno P. Characterization of the gastric microbiota in a pediatric population according to Helicobacter pylori status. In: Pediatric Infectious Disease Journal. Vol 36. Lippincott Williams and Wilkins; 2017:173-8.

31. Parsons BN, Ijaz UZ, D'Amore R. Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of Helicobacter pylori-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use. Blanke SR, ed. PLOS Pathog. 2017;13(11):e1006653.

32. Fallone CA, Barkun AN, Gottke MU. Association of Helicobacter pylori genotype with gastroesophageal reflux disease and other upper gastrointestinal diseases. Am J Gastroenterol. 2000;95(3):659-69.

33. Labenz J, Blum AL, Bayerdorffer E, Meining A, Stolte M, Borsch G. Curing Helicobacter pylori infection in patients with duodenal ulcer may provoke reflux esophagitis. Gastroenterology. 1997;112(5):1442-7.

34. Schwizer W, Thumshirn M, Dent J. Helicobacter pylori and symptomatic relapse of gastro-oesophageal reflux disease: a randomised controlled trial. Lancet. 2001;357(9270):1738-42.

35. Potamitis GS, Axon ATR. Helicobacter pylori and Nonmalignant Diseases. Helicobacter. 2015;20:26-29.

36. Ghoshal UC, Chourasia D. Gastroesophageal Reflux Disease and : What May Be the Relationship? J Neurogastroenterol Motil. 2010;16(3):243-50.

37. Werdmuller BF, Loffeld RJ. Helicobacter pylori infection has no role in the pathogenesis of reflux esophagitis. Dig Dis Sci. 1997;42(1):103-5.

38. Vakil N, Hahn B, McSorley D. Recurrent symptoms and gastro-oesophageal reflux disease in patients with duodenal ulcer treated for Helicobacter pylori infection. Aliment Pharmacol Ther. 2000;14(1):45-51.

39. Kuipers EJ, Nelis GF, Klinkenberg-Knol EC. Cure of Helicobacter pylori infection in patients with reflux oesophagitis treated with long term omeprazole reverses gastritis without exacerbation of reflux disease: Results of a randomised controlled trial. Gut. 2004;53(1):12-20.

40. Uno Y. GERD by colonic fermentation. Neurogastroenterol Motil. 2020;32(3).

41. Piche T, Des Varannes SB, Sacher-Huvelin S, Holst JJ, Cuber JC, Galmiche JP. Colonic fermentation influences lower esophageal sphincter function in gastroesophageal reflux disease. Gastroenterology. 2003;124(4):894-902.

42. Ropert A, Cherbut C, Roze C. Colonic fermentation and proximal gastric tone in humans. Gastroenterology. 1996;111(2):289-96.

43. Larraufie P, Martin-Gallausiaux C, Lapaque N. SCFAs strongly stimulate PYY production in human enteroendocrine cells. Sci Rep. 2018;8(1).

44. Shaheen NJ, Falk GW, Iyer PG, Gerson LB. ACG Clinical Guideline: Diagnosis and Management of Barrett's Esophagus. Am J Gastroenterol. 2016;111(1):30-50.

45. Naini B V, Souza RF, Odze RD. Barrett's esophagus: A comprehensive and contemporary review for pathologists. Am J Surg Pathol. 2016;40(5):e45-e66.

46. Pei ZH, Yang L, Peek RM, Levine SM, Pride DT, Blaser MJ. Bacterial biota in reflux esophagitis and Barrett's esophagus. World J Gastroenterol. 2005;11(46):7277-83.

47. Quante M, Bhagat G, Abrams JA. Bile acid and inflammation activate gastric cardia stem cells in a mouse model of barrett-like metaplasia. Cancer Cell. 2012;21(1):36-51.

48. Münch NS, Fang HY, Ingermann J. High-Fat Diet Accelerates Carcinogenesis in a Mouse Model of Barrett's Esophagus via Interleukin 8 and Alterations to the Gut Microbiome. Gastroenterology. 2019;157(2):492-506.e2.

49. Snider EJ, Compres G, Freedberg DE. Barrett's esophagus is associated with a distinct oral microbiome. Clin Transl Gastroenterol. 2018;9(3).

50. Yang L, Chaudhary N, Baghdadi J, Pei Z. Microbiome in reflux disorders and esophageal adenocarcinoma. Cancer J (United States). 2014;20(3):207-10.

51. Liu N, Ando T, Ishiguro K. Characterization of bacterial biota in the distal esophagus of Japanese patients with reflux esophagitis and Barrett's esophagus. BMC Infect Dis. 2013;13(1):130.

52. Konturek PC, Nikiforuk A, Kania J, Raithel M, Hahn EG, Mühldorfer S. Activation of NFkappaB represents the central event in the neoplastic progression associated with Barrett's esophagus: a possible link to the inflammation and overexpression of COX-2, PPARgamma and growth factors. Dig Dis Sci. 2004;49(7-8):1075-83.

53. Neto AG, Whitaker A, Pei Z. Microbiome and potential targets for chemoprevention of esophageal adenocarcinoma. Semin Oncol. 2016;43(1):86-96.

54. Segata N, Haake SK, Mannon P. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genome Biol. 2012;13(6):R42.

55. Choksi Y, Vaezi MF. Preliminary esophageal microbiome studies prompt important scientific questions editorial. Clin Transl Gastroenterol. 2018;9(5).

56. Abbas G, Krasna M. Overview of esophageal cancer. Ann Cardiothorac Surg. 2017;6(2):131-6.

57. Abnet CC, Arnold M, Wei WQ. Epidemiology of Esophageal Squamous Cell Carcinoma. Gastroenterology. 2018;154(2):360-73.

58. Kamangar F, Chow WH, C. Abnet C, M. Dawsey S. Environmental Causes of Esophageal Cancer. Gastroenterol Clin North Am. 2009;38(1):27-57.

59. Engel LS, Chow WH, Vaughan TL. Population attributable risks of esophageal and gastric cancers. J Natl Cancer Inst. 2003;95(18):1404-13.

60. Susan G. Urba M. Adenocarcinoma of the Esophagus: Risk Factors and Prevention. 2000.

61. Bhat S, Coleman HG, Yousef F. Risk of malignant progression in Barrett's Esophagus patients: Results from a large population-based study. J Natl Cancer Inst. 2011;103(13):1049-57.

62. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. J Natl Cancer Inst. 2005;97(2):142-6.

63. Yang CS, Chen X, Tu S. Etiology and Prevention of Esophageal Cancer. Gastrointest Tumors. 2016;3(1):3-16.

64. Maghsudlu M, Farashahi Yazd E. Heat-induced inflammation and its role in esophageal cancer. J Dig Dis. 2017;18(8):431-44.

65. Varela-Rey M, Woodhoo A, Martinez-Chantar ML, Mato JM, Lu SC. Alcohol, DNA methylation, and cancer. Alcohol Res Curr Rev. 2012;35(1):25-35.

66. Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. Nat Rev Cancer. 2007;7(8):599-612.

67. Li X, Li H, Dong X. Expression of NF-κB and TLR-4 is associated with the occurrence, progression and prognosis of esophageal squamous cell carcinoma. Int J Clin Exp Pathol. 2018;11(12):5850-9.

68. Chen X, Winckler B, Lu M. Oral Microbiota and Risk for Esophageal Squamous Cell Carcinoma in a High-Risk Area of China. Wei Q-Y, ed. PLoS One. 2015;10(12):e0143603.

69. Peters BA, Wu J, Pei Z. Oral microbiome composition reflects prospective risk for esophageal cancers. Cancer Res. 2017;77(23):6777-87.

70. Yu G, Gail MH, Shi J. Association between upper digestive tract microbiota and cancer-predisposing states in the esophagus and stomach. Cancer Epidemiol Biomarkers Prev. 2014;23(5):735-41.

71. Narikiyo M, Tanabe C, Yamada Y. Frequent and preferential infection of Treponema denticola, Streptococcus mitis, and Streptococcus anginosus in esophageal cancers. Cancer Sci. 2004;95(7):569-74.

72. Michaud DS, Fu Z, Shi J, Chung M. Periodontal Disease, Tooth Loss, and Cancer Risk. Epidemiol Rev. 2017;39(1):49-58.

73. Tateda M, Shiga K, Saijo S. Streptococcus anginosus in head and neck squamous cell carcinoma: implication in carcinogenesis. Int J Mol Med. 2000;6(6):699-703.

74. Malinowski B, Węsierska A, Zalewska K. The role of Tannerella forsythia and Porphyromonas gingivalis in pathogenesis of esophageal cancer. Infect Agent Cancer. 2019;14(1).

75. Lv J, Guo L, Liu JJ, Zhao HP, Zhang J, Wang JH. Alteration of the esophageal microbiota in Barrett's esophagus and esophageal adenocarcinoma. World J Gastroenterol. 2019;25(18):2149-61.

76. Elliott DRF, Walker AW, O'Donovan M, Parkhill J, Fitzgerald RC. A non-endoscopic device to sample the oesophageal microbiota: a case-control study. Lancet Gastroenterol Hepatol. 2017;2(1):32-42.

77. Zaidi AH, Kelly LA, Kreft RE. Associations of microbiota and toll-like receptor signaling pathway in esophageal adenocarcinoma. BMC Cancer. 2016;16(1):52.

78. Morris CD, Armstrong GR, Bigley G, Green H, Attwood SEA. Cyclooxygenase-2 Expression in The Barrettęs Metaplasia–Dysplasia–Adenocarcinoma Sequence. Am J Gastroenterol. 2001;96(4):990-6.

79. Gallimidi AB, Fischman S, Revach B. Periodontal pathogens Porphyromonas gingivalis and Fusobacterium nucleatum promote tumor progression in an oral-specific chemical carcinogenesis model. Oncotarget. 2015;6(26):22613-23.

80. Sepehr A, Kamangar F, Fahimi S, Saidi F, Abnet CC, Dawsey SM. Poor oral health as a risk factor for esophageal squamous dysplasia in northeastern Iran. Anticancer Res. 2005;25(1B):543-6.

81. Schmidt BL, Kuczynski J, Bhattacharya A. Changes in abundance of oral microbiota associated with oral cancer. PLoS One. 2014;9(6).

82. Jovanovic A, van der Tol IGH, Kostense PJ. Second respiratory and upper digestive tract cancer following oral squamous cell carcinoma. Eur J Cancer Part B Oral Oncol. 1994;30(4):225-9.

83. Simple M, Suresh A, Das D, Kuriakose MA. Cancer stem cells and field cancerization of Oral squamous cell carcinoma. Oral Oncol. 2015;51(7):643-51.

84. Ahn J, Sinha R, Pei Z. Human gut microbiome and risk for colorectal cancer. J Natl Cancer Inst. 2013;105(24):1907-11.

85. Nasrollahzadeh D, Malekzadeh R, Ploner A. Variations of gastric corpus microbiota are associated with early esophageal squamous cell carcinoma and squamous dysplasia. Sci Rep. 2015;5.

86. Homann N, Kärkkäinen P, Koivisto T, Nosova T, Jokelainen K, Salaspuro M. Effects of acetaldehyde on cell regeneration and differentiation of the upper gastrointestinal tract mucosa. J Natl Cancer Inst. 1997;89(22):1692-7.

87. Homann N, Tillonen J, Rintamäki H, Salaspuro M, Lindqvist C, Meurman JH. Poor dental status increases acetaldehyde production from ethanol in saliva: A possible link to increased oral cancer risk among heavy drinkers. Oral Oncol. 2001;37(2):153-8.

88. Matejcic M, Gunter MJ, Ferrari P. Alcohol metabolism and oesophageal cancer: a systematic review of the evidence. Carcinogenesis. 2017;38(9):859-72.

89. Bull-Otterson L, Feng W, Kirpich. Metagenomic Analyses of Alcohol Induced Pathogenic Alterations in the Intestinal Microbiome and the Effect of Lactobacillus rhamnosus GG Treatment. PLoS One. 2013;8(1).

90. Ying W, Jing T, Bing C, Baifang W, Dai Z, Bingyuan W. Effects of alcohol on intestinal epithelial barrier permeability and expression of tight junction-associated proteins. Mol Med Rep. 2014;9(6):2352-6.

91. Lippai D, Bala S, Catalano D, Kodys K, Szabo G. Micro-RNA-155 Deficiency Prevents Alcohol-Induced Serum Endotoxin Increase and Small Bowel Inflammation in Mice. Alcohol Clin Exp Res. 2014;38(8):2217-24.

92. Collins SM. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. Gastroenterology. 1996;111(6):1683-99.

93. Isomoto H, Nishi Y, Kanazawa Y. Immune and inflammatory responses in GERD and lansoprazole. J Clin Biochem Nutr. 2007;41(2):84-91.

94. Rieder F, Cheng L, Harnett KM. Gastroesophageal Reflux Disease-Associated Esophagitis Induces Endogenous Cytokine Production Leading to Motor Abnormalities. Gastroenterology. 2007;132(1):154-65.

95. Pajecki D, Zilberstein B, dos Santos MAA. Megaesophagus microbiota: a qualitative and quantitative analysis. J Gastrointest Surg. 6(5):723-9.

96. Pajecki D, Zilberstein B, Cecconello I, Dos Santos MAA, Yagi OK, Gama-Rodrigues JJ. Larger amounts of nitrite and nitrate-reducing bacteria in megaesophagus of Chagas' disease than in controls. J Gastrointest Surg. 2007;11(2):199-203.

97. Ge X, Zhao W, DIng C. Potential role of fecal microbiota from patients with slow transit constipation in the regulation of gastrointestinal motility. Sci Rep. 2017;7(1).

98. Cherbut C. Motor effects of short-chain fatty acids and lactate in the gastrointestinal tract. Proc Nutr Soc. 2003;62(1):95-9.

99. Talotta R, Atzeni F, Ditto MC, Gerardi MC, Sarzi-Puttini P. The Microbiome in Connective Tissue Diseases and Vasculitides: An Updated Narrative Review. J Immunol Res. 2017;2017:6836498.

100. Manetti M, Milia AF, Benelli G, Messerini L, Matucci-Cerinic M, Ibba-Manneschi L. The gastric wall in systemic sclerosis patients: A morphological study. Ital J Anat Embryol. 2010;115(1-2):115-21.

101. Andréasson K, Alrawi Z, Persson A, Jönsson G, Marsal J. Intestinal dysbiosis is common in systemic sclerosis and associated with gastrointestinal and extraintestinal features of disease. Arthritis Res Ther. 2016;18(1):278.

102. Volkmann ER, Hoffmann-Vold AM, Chang YL. Systemic sclerosis is associated with specific alterations in gastrointestinal microbiota in two independent cohorts. BMJ Open Gastroenterol. 2017;4(1):e000134.

103. Patrone V, Puglisi E, Cardinali M. Gut microbiota profile in systemic sclerosis patients with and without clinical evidence of gastrointestinal involvement. Sci Rep. 2017;7(1):14874.

104. Bellocchi C, Fernández-Ochoa Á, Montanelli G. Microbial and metabolic multi-omic correlations in systemic sclerosis patients. Ann N Y Acad Sci. 2018;1421(1):97-109.

105. Dellon ES, Gonsalves N, Hirano I, Furuta GT, Liacouras CA, Katzka DA. ACG clinical guideline: Evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). Am J Gastroenterol. 2013;108(5):679-92.

106. Torrijos EG, Gonzalez-Mendiola R, Alvarado M. Eosinophilic esophagitis: Review and update. Front Med. 2018;5(OCT).

107. Spechler SJ. Eosinophilic esophagitis: novel concepts regarding pathogenesis and clinical manifestations. J Gastroenterol. 2019;54(10):837-44.

108. Simon D, Straumann A, Schoepfer AM, Simon H-U. Current concepts in

eosinophilic esophagitis. Allergo J Int. 2017;26(7):258-66.

109. Sherrill JD, Kiran KC, Blanchard C. Analysis and expansion of the eosinophilic esophagitis transcriptome by RNA sequencing. Genes Immun. 2014;15(6):361-9.

110. Rieder F, Nonevski I, Ma J. T-helper 2 cytokines, transforming growth factor β1, and eosinophil products induce fibrogenesis and alter muscle motility in patients with eosinophilic esophagitis. Gastroenterology. 2014;146(5):1266-77.e1-9.

111. Harris JK, Fang R, Wagner BD. Esophageal microbiome in eosinophilic esophagitis. PLoS One. 2015;10(5).

112. Benitez AJ, Hoffmann C, Muir AB. Inflammation-associated microbiota in pediatric eosinophilic esophagitis. Microbiome. 2015;3(1):23.

113. Shin NR, Whon TW, Bae JW. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015;33(9):496-503.

114. Hiremath G, Shilts MH, Boone HH. The Salivary Microbiome Is Altered in Children With Eosinophilic Esophagitis and Correlates With Disease Activity. Clin Transl Gastroenterol. 2019;10(6):e00039.

115. Kaur S, Kaur S. Bacteriocins as potential anticancer agents. Front Pharmacol. 2015;6(NOV).

116. Kumariya R, Garsa AK, Rajput YS, Sood SK, Akhtar N, Patel S. Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. Microb Pathog. 2019;128:171-7.

117. Lobo LA, Benjamim CF, Oliveira AC. The interplay between microbiota and inflammation: lessons from peritonitis and sepsis. Clin Transl Immunol. 2016;5(7):e90.

118. Sorini C, Cardoso RF, Gagliani N, Villablanca EJ. Commensal bacteria-specific CD4+T cell responses in health and disease. Front Immunol. 2018;9(NOV):2667.

119. Siracusa F, Schaltenberg N, Villablanca EJ, Huber S, Gagliani N. Dietary habits and intestinal immunity: From food intake to CD4+TH cells. Front Immunol. 2019;10(JAN):3177.

120. Selling J, Swann P, Madsen LR, Oswald J. Improvement in Gastroesophageal Reflux Symptoms From a Food-grade Maltosyl-isomaltooligosaccharide Soluble Fiber Supplement: A Case Series. Integr Med (Encinitas). 2018;17(5):40-2.

121. Gomi A, Yamaji K, Watanabe O. Bifidobacterium bifidum YIT 10347 fermented milk exerts beneficial effects on gastrointestinal discomfort and symptoms in healthy adults: A double-blind, randomized, placebo-controlled study. J Dairy Sci. 2018;101(6):4830-41.

122. Waller PA, Gopal PK, Leyer GJ. Dose-response effect of Bifidobacterium lactis HN019 on whole gut transit time and functional gastrointestinal symptoms in adults. Scand J Gastroenterol. 2011;46(9):1057-64.

123. Nakae H, Tsuda A, Matsuoka T, Mine T, Koga Y. Gastric microbiota in the functional dyspepsia patients treated with probiotic yogurt. BMJ Open Gastroenterol. 2016;3(1):e000109.

124. Ohtsu T, Takagi A, Uemura N. The Ameliorating Effect of Lactobacillus gasseri OLL2716 on Functional Dyspepsia in Helicobacter pylori -Uninfected Individuals: A Randomized Controlled Study. Digestion. 2017;96(2):92-102.

125. Cheng J, Ouwehand AC. Gastroesophageal reflux disease and probiotics: A systematic review. Nutrients. 2020;12(1).

126. Sun QH, Wang HY, Sun SD, Zhang X, Zhang H. Beneficial effect of probiotics supplements in reflux esophagitis treated with esomeprazole: A randomized controlled trial. World J Gastroenterol. 2019;25(17):2110-21.

127. Zmora N, Zilberman-Schapira G, Suez J. Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. Cell. 2018;174(6):1388-405.e21.

128. Rao SSC, Bhagatwala J. Small Intestinal Bacterial Overgrowth. Clin Transl Gastroenterol. 2019;10(10):e00078.

129. Young Chang J, Kim S-E, Hun Kim T. Emergence of rifampin-resistant staphylococci after rifaximin administration in cirrhotic patients. 2017.

130. Mcdonald LC, Gerding DN, Johnson S. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis Clin Pract Guidel Clostridium difficile Infect • CID. 2018;1.

131. Okuda KI, Zendo T, Sugimoto S. Effects of bacteriocins on methicillin-resistant Staphylococcus aureus biofilm. Antimicrob Agents Chemother. 2013;57(11):5572-9.

132. Kommineni S, Bretl DJ, Lam V. Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. Nature. 2015;526(7575):719-22.

133. Fahim HA, Khairalla AS, El-Gendy AO. Nanotechnology: A valuable strategy to improve bacteriocin formulations. Front Microbiol. 2016;7(SEP):1385.