ANTICANCER RESEARCH *35*: 6365-6372 (2015)

Review

Increased Production of Lysozyme Associated with Bacterial Proliferation in Barrett's Esophagitis, Chronic Gastritis, Gluten-induced Atrophic Duodenitis (Celiac Disease), Lymphocytic Colitis, Collagenous Colitis, Ulcerative Colitis and Crohn's Colitis

CARLOS A. RUBIO

Department of Pathology, Karolinska Instutute and University Hospital, Stockholm, Sweden

Abstract. *The mucosa of the esophagus, the stomach, the small intestine, the large intestine and rectum are unremittingly challenged by adverse micro-environmental factors, such as ingested pathogenic and non-pathogenic bacteria, and harsh secretions with digestive properties with disparate pH, as well as bacteria and secretions from upstream GI organs. Despite the apparently inauspicious mixture of secretions and bacteria, the normal GI mucosa retains a healthy state of cell renewal. To by-pass the tough microenvironment, the epithelia of the GI react by speeding-up cell exfoliation, by increasing peristalsis, eliminating bacteria through secretion of plasma cell-immunoglobulins and by increasing production of natural antibacterial enzymes (lysozyme) and host defense peptides (defensin-5). Lysozyme was recently found up-regulated in Barrett's esophagitis, in chronic gastritis, in gluten-induced atrophic duodenitis (celiac disease), in collagenous colitis, in lymphocytic colitis and in Crohn's colitis. This up-regulation is a response directed towards the special types of bacteria thriving in the microenvironment in each of the aforementioned clinical inflammatory maladies. The purpose of that up-regulation is to protect the mucosa affected by the ongoing chronic inflammation. Bacterial antibiotic resistance continues to exhaust our supply of effective antibiotics. The future challenge is how to solve the increasing menace of bacterial resistance to anti-bacterial drugs. Further research on natural anti-bacterial enzymes such as lysozyme, appears mandatory.*

Correspondence to: C.A. Rubio, MD, Ph.D., Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, 17176, Stockholm, Sweden. Fax: +46 851774524, e-mail: Carlos.Rubio@ki.se

Key Words: Lysozyme, chronic inflammation, esophagus, stomach, duodenum, colon, review.

The cells that line the mucosa of the human gastrointestinal (GI) tract are continuously exposed to adverse micro-environmental conditions, such as digestive juices of different pH, a wide variety of active natural enzymes and large amount of bacteria. The density of the bacterial flora in the GI tract is huge; it varies from 10^3/ml near the gastric outlet to 10^{10}/ml at the ileo-cecal valve to 10^{11} to 10^{12}/ml in the colon. The total microbial population (aproximatelly 10^{14}) exceeds the total number of cells in the GI tract. About 500 to 1,000 different species exist, a biomass that weights about 1.5 kg (1). Calculating an average genome size for 1,000 *Escherichia coli* species, the number of genes in this microbiome exceeds the total number of human genes by a factor of 100. These bacteria procreate in a luminal bolus that transports a potion of secretions from various organs carrying the extracellular glycoprotein glycocalix (2). Moreover, secretions with digestive properties and cocktails of bacteria from the upper GI tract challenge the epithelia from downstream organs. These unfavourable conditions would be detrimental for unprotected GI cells.

However, despite the inauspicious mixture of harmful secretions and bacteria, the normal GI mucosa retains a healthy state of cell renewal. To counteract the aggressive microenvironment, GI epithelia react by speeding cell exfoliation (GI mucosa has a turnover time of 2 to 3 days), by increasing peristalsis, by eliminating bacteria through secretion of plasma cell-immunoglubulins and by increasing production of natural antibacterial compounds, such as defensin-5 and lysozyme.

During a deliberate search for medical antibiotics, Alexander Fleming (3) discovered lysozyme, one of the

natural defence substances against infection. Lysozyme, also known as muramidase or N-acetylmuramide glycanhydrolase, is a family of enzymes (EC 3.2.1.17) that damage bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins (4). Lysozyme is encoded by the *LYZ* gene (5). Lysozyme is an ancient enzyme whose origin goes back an estimated 400 to 600 million years (6). Linkage studies indicate that the lysozyme M and P genes are present on the same chromosome and calculations from both partial protein sequence and phylogenetic data indicate that the duplication that gave rise to those genes occurred about 50 million years ago (5). It should be pointed-out that lysozyme is only a generic name (*v. gr*. lysozyme c is a superfamily composed of 88 distinct lysozymes).

Recently, lysozyme was found up-regulated in many organs of the GI undergoing chronic inflammation, such as in Barrett's esophagitis, chronic gastritis, gluten-induced atrophic duodenitis (celiac disease), collagenous colitis, lymphocytic colitis, ulcerative colitis (UC) and Crohn's colitis (7-12), strongly suggesting that the associated bacterial flora plays an important role in the up-regulation of this antimicrobial enzyme.

Barrett's Esophagitis

Following protracted gastric reflux the normal squamous-cell epithelium of the distal esophagus may undergo columnar-lined (metaplastic) transformation both in humans (13), and in non-human primates (14, 15). The metaplastic transformation in Barrett's esophagus includes accessory glands of oxyntic type and/or pyloric type with or without intercalated goblet cells (16), known as specialized epithelium or intestinal metaplasia (IM) (17). The British Society of Gastroenterology (BSG) (18) defined Barrett's esophagus as a columnar-lined oesophagus on biopsies taken from endoscopical areas suggestive of Barrett's oesophagus. Thus, the presence of GC is not a *sine qua non* requirement for the diagnosis of Barrett's oesophagus (19, 20).

Patients with gastro-esophageal reflux often receive proton pump inhibitor (PPI) medication. The reduction of gastric acid secretion by PPI encourages bacterial growth in Barrett's esophagitis triggering, thereby, increased production of nitrosamines with secondary epithelial damage (21).

Bacteria in Barrett's esophagitis. Several studies have demonstrated that special bacteria are more often present in esophageal biopsies with Barrett's esophagus than in those without Barrett's esophagus (22-26). Esophageal microbiomes have been classified into two types: type I microbiome, dominated by the genus *Streptococcus*, concentrated in the phenotypically normal oesophagus, and type II microbiome containing a greater proportion of Gram-negative anaerobes/microaerophiles primarily found in oesophagitis and Barrett's esophagus (25). In gastro-esophageal reflux, residential bacterial populations contain 21 distinct species including *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* (23). In a more recent study with esophageal biopsies and aspirates, Mc Farlane *et al*. (24) found in the mucosa of the Barrett's esophagus 46 bacterial species belonging to 16 genera with unique levels of *Campylobacter consisus* and *C. rectus*. Taken together, these microbiological findings denote a close association between the occurrence of columnar-lined esophageal mucosa and the proliferation of abnormal bacteria in the esophageal microenvironment. More recently, Liu *et al*. found *Firmicutes* (55%), *Proteobacteria* (20%), *Bacteroidetes* (14%), *Fusobacteria* (9%), and *Actinobacteria* (2%), in analysis of 138 16S rDNA sequences from 240 clones of 6 cases of Barrett's esophagus (26). The oesophageal bacterial composition differed in the normal esophagus, in reflux oesophagitis, and in Barrett' s esophagus. Diverse bacterial communities may be associated with esophageal disease.

Lysozyme is up-regulated in Barrett's esophagitis. An increased lysozyme immunoreactivity is found in Barrett's esophagus; in the surface columnar epithelium, in the columnar epithelium of the pits of the glands, in goblet cells as well as in Paneth cells in cases with intestinal metaplasia (27). In some goblet cells, lysozyme is slightly expressed. This phenomenon might be due to a prior goblet cell-discharge of lysozyme-rich intracellular mucus into the lumen. Lysozyme is not expressed in parietal (oxyntic) cells, neither in Barrett's esophagus, nor in controls (27). These findings indicate that in Barrett's esophagus lysozyme is up-regulated in Paneth cells and in mucus-secreting cells. When compared to controls, lysozyme is up-regulated in all three Barrett's mucosal phenotypes (27).

Intestinal metaplasia in Barrett's esophagus is not a transformation into intestinal cells, but a phenomenon of reconstruction by migrant stem cells of bone marrow origin adapting to the hostile microenvironment (28). Circulating bone marrow cells would engraft into ulcerated mucosal areas affected by on-going chronic inflammation. In a murine model multi-potential progenitor cells of bone marrow origin were found to contribute to the intestinal metaplastic epithelium in the oesophagus (28). Stem cells in the esophagus might be instrumental in the molecular cross-talk between intraluminal bacterial flora and the production of lysozyme (29); signals released from the particular bacterial flora might induce stem cells in the Barrett's esophagus to generate differentiated cells rich in the anti-microbial enzyme lysozyme.

Gastritis

Based on its topographic localization and etiological cause(s), chronic gastritis is classified into antrum predominant gastritis (environmental or type B gastritis) and corpus predominant gastritis (autoimmune or type A gastritis) (30). A*ntral predominant chronic gastritis*. In 1983, Warren and Marshal (31) discovered *Helicobacter pylori*, the most common etiological bacteria of active antrum gastritis. Approximately 50% of the world's population are infected with these bacteria, according to studies (32). Five years after, Correa postulated that the *H. pylori* is the principal agent that set aflame the cascade of histological events that telescope from chronic gastritis to carcinoma through mucosal atrophy, intestinal metaplasia and epithelial dysplasia (33).

Bacteria in antral predominant, active chronic gastritis. Years ago, Giannella *et al*. (34) studied *in vitro* the bactericidal activity of the normal and achlorhydric gastric juice obtained from various patients. When the pH was less than 4.0, 99.9% of bacteria were killed within 30 min *in vitro*, indicating that the gastric bactericidal barrier is primarily pH-hydrochloric-acid dependent, with other constituents of gastric juice contributing little, if any, to destruction of microorganisms (34). Consequently, in acid-deficient stomachs, a mechanism other than gastric acidity counteracts the ingested bacteria. Many investigators demonstrated patchy (multifocal) gastric mucosal inflammation in *H. pylori*-infected stomachs, first at the *incisura angularis* spreading subsequently to the antrum and less frequently to the corpus (35). The host reacts to *H. pylori* by increasing the number of T- and B-lymphocytes, followed by polymorphonuclear leucocytic infiltration aiming to phagocytize the bacteria. Bacteria adhesion molecules encourage attachment to the foveolar cells, and bacteria proteases and urease damage the gastric epithelium. The next stage is the destruction of glands by $CD3^+$ T lymphocytes (36). On the other hand, not all patients with *H. pylori* infection develop gastritis, since only those strains that possess the cytoxin-associated gene pathogenicity island are able to secrete a toxin that severely injures gastric mucosa. The specific significance of *H. pylori* in the aetiology of atrophic gastritis has recently been questioned (37, 38) since gastric mucosal inflammation might also be caused by bacteria other than *H. pylori*, by virus, by *Candida albicans*, by excessive alcohol use, by chronic vomiting, retrograde bile reflux, autoantibodies, stress, aspirin and other anti-inflammatory drugs such as NSAID.

Corpus predominant (autoimmune) chronic gastritis. This gastritis phenotype is an inflammatory disease of the gastric mucosa triggered by autoantibodies to parietal cells and intrinsic factor (39-41). In autoimmune gastritis the inflammatory infiltrates, and glandular atrophy with or without intestinal metaplasia are restricted to the oxyntic mucosa and do not compromise the antral mucosa (41). Autoimmune gastritis is accompanied by a neuroendocrine enterochromaffin-like cell hyperplasia in the corpus. All parietal cells exhibit specific monoclonal antibodies, but in some patients the gastric mucosa is microscopically intact. In advanced forms, the body mucosa is inflamed and shows extensive absence of glands (gastric atrophy). The body mucosa is eventually replaced by pseudo-pyloric metaplasia (due to hyperplasia of the mucous neck cell). Typically indolent enterochromaffin-like cell nodular hyperplasia and multiple carcinoid tumors may develop in the atrophic body mucosa, whereas the mucosa of the antrum remains relatively spared.

Bacteria in autoimmune gastritis. The true role played by *H. pylori* infection in autoimmune gastritis remains controversial. Due to progressive mucosal atrophy microbial diversity increases with reduced acidity (42). By applying temporal temperature gradient gel electrophoresis and 16S rRNA sequencing, Monstein *et al*. demonstrated in the stomach, particular microbes (other than *H. pylori*), such as *Enterococcus, Pseudomonas, Streptococcus, Staphylococcus* and *Stomatococcus* (43). Using large-scale 16S rRNA sequencing 128 phylotypes from 8 phyla were identified (44), thus confirming the complexity of microbiota in the gastric mucosa. In patients with antral gastritis Li *et al*. found 133 phylotypes from eight bacterial phyla as well as 11 *Streptococcus* phylotypes, including *Firmicutes* phylum and *Streptococcus* genus from cultivated biopsies (45). In the absence of *H. pylori*, other bacterial groups/species seem to trigger gastritis development. Unfortunately no patients with autoimmune chronic gastritis were included in Li *et al*. (45) studies.

Fundic Gland Polyps

Fundic gland polyps are small (≤5 mm) nodules of the gastric mucosa characterized by microcysts lined with parietal, chief cells and occasional mucous foveolar cells (46-48), usually found in patients with hereditary diseases such as familial adenomatous polyposis/Gartner's syndrome and juvenile polyposis. FGP are also seen in patients with non-hereditary (*i.e*. sporadic) gastric disorders or receiving proton-pump inhibitor. *H. pylori* does not proliferate in fundic gland polyps (46-48). The cause(s) for this lack of association have remained elusive, but it appears related to the up-regulation of lysozyme.

Lysozyme is up-regulated in chronic gastritis, intestinal metaplasia, autoimmune gastritis and fundic-gland polyps. In chronic gastritis lysozyme is up-regulated in the neck region of

the oxyntic mucosa, in the antro-pyloric glands and in the surface-foveolar epithelium of the oxyntyc mucosa (49). In cases with intestinal metaplasia, lysozyme is up-regulated in goblet cells and in Paneth cells. In cases with autoimmune gastritis, lysozyme is up-regulated in pseudopyloric glands. The increased lysozyme expression in goblet cells of gastric intestinal metaplasia appears to be a phenomenon unrelated to the presence of Paneth cells, as lysozyme is similarly overexpressed in cases with complete intestinal metaplasia (*i.e.* having Paneth cells) and with incomplete intestinal metaplasia (*i.e.* without Paneth cells). As pseudo-pyloric glands are generated by hyperplasia of the mucous neck cells it is not surprising that these cells retain the characteristics of the mucus neck cells, namely lysozyme expression (8). Human defensin 5 secreted by Paneth cells in the small intestine may also regulate and maintain microbial balance in the intestinal lumen in contrast to the non-metaplastic atrophic gastric mucosa that does not provide a similar defensive reaction.

Intestinal metaplasia might evolve following a mucosal insult that affects the stem cells of the crypts of Lieberkhün (28). Our studies (8, 49) strongly suggest that gastric intestinal metaplasia and gastric atrophy are two different biological processes, atrophy being the result of the local destruction of glands by the chronic inflammation, and intestinal metaplasia, the consequence of an adaptive enzymatic up-regulation aimed to protect the mucosa from proliferating bacteria.

Years ago, Shousa *et al.* observed a significantly higher prevalence of intestinal metaplasia in gastric biopsies from British patients than in Yemeni patients (50). In comparative studies of 1,675 gastric biopsies and gastrectomy specimens having chronic gastritis we found intestinal metaplasia in 59% of Japanese patients (51), in 50% of Italian patients (39), in 32% of Swedish patients (51), and in 13% of Mexican patients (52), suggesting that environmental factors might trigger intestinal metaplasia in chronic gastritis. Importantly, it has been repeatedly demonstrated that *H. pylori* is absent in areas with intestinal metaplasia or with pseudo-pyloric metaplasia. Hence, it appears safe to postulate that the aim of lysozyme over-production in intestinal metaplasia and in pseudo-pyloric metaplasia might be to eradicate luminal proliferating bacteria in acid-deficient stomachs.

In fundic gland polyps, lysozyme is up-regulated in the surface epithelium, the foveolar pits and the cells that partly or entirely cover dilated glands (47, 49). The over-production of lysozyme by the fundic gland polyp epithelium concurs with the absence of H.pylori in these lesions (49).

Gluten-induced Atrophic Duodenitis (Celiac Disease)

Celiac disease is a common immune-mediated condition in the proximal small intestine often leading to mucosal atrophy generated by a permanent intolerance to cereal gluten proteins in genetically predisposed individuals (53). In most Western countries the prevalence of diagnosed celiac disease in children is 0.5-1% (54). Celiac disease is the second most common chronic disease in Swedish children with an incidence of 3% (55).

Bacteria in celiac disease. In later years great attention has been attributed on the abnormal microbiota present in the duodenum in patients with celiac disease. Bifido bacterium, Bacteroides vulgatus, *Escherichia coli* and rod-shape bacteria attached to the intestinal epithelium were found to be higher in patients with celiac disease than in controls, whereas *B. bacterium adolescentis*/*B. bacterium animalis lactis* were more prevalent in patients with active celiac disease than in patients with treated celiac disease/control patients (56- 59).

Lysozyme is up-regulated in gluten-induced atrophic duodenitis (celiac disease). In normal duodenal mucosa Paneth cells, located at the base of the crypts, produce lysozyme. In coeliac disease, lysozyme is up-regulated in goblet cells and in the mucus-metaplasia found in dilated crypts, a phenomenon more apparent in the *bulbus duodeni* (10). Rationally, there might exist a critical limit for the number of Paneth cells that can be housed at the base of single crypts in coeliac disease. It is not inconceivable that the lysozyme-rich mucus metaplasia mirror stem cell adaptation to the signals generated by the alien pathogenic bacteria present in the duodenal microenvironment (60).

Collagenous Colitis, Lymphocytic Colitis, Ulcerative Colitis and Crohn's Colitis

In 1976 the Swedish pathologist CG Lindström reported the presence of a sub-epithelial amorphous band in the colonic mucosa in a patient having chronic watery diarrhea and grossly normal colonoscopy (61); he called this setting collagenous colitis. In 1989 Giardelo *et al.* (62) found an increased number of intraepithelial lymphocytes in the superficial epithelium of the colon in patients having watery diarrhea and grossly normal colonoscopy, and proposed the term lymphocytic colitis for this type of microscopic colitis. After an initial rise during 1980s and early 1990s, the annual incidence of collagenous colitis and lymphocytic colitis in Sweden has been stable during the past 15 years, about 5/100,000 inhabitants for each disorder (63).

Bacteria in microscopic colitis. Our understanding of a possible alien bacteria flora in microscopic colitis is poor. In collagenous colitis *Firmicutes* and *Bacteroidetes* were found to dominate the microbiota with seven phylotypes among 50% of the clones: *B. cellulosilyticus, B. caccae, B. thetaiotaomicron, B. uniformis, B. dorei, B. spp.* and clones showing similarity to Clostridium clostridioforme (64). More

recently, Helal *et al*. found an association between *E. coli* and lymphocytic colitis (65).

Bacteria in inflammatory bowel disease. Our understanding of a possible alien bacteria flora in inflammatory bowel disease is less clear (66-77).

Bacteria in ulcerative colitis. In ulcerative colitis, bacterial diversity is reduced, including Clostridium groups IV, XIVa (*Faecalibacterium prausnitzii*) and Bifidobacteria and lactobacillia. On the other hand, *C. difficile* is increased. *In vitro* batch cultures of gut microbiota from healthy and ulcerative colitis subjects suggest that sulphate-reducing bacteria levels are raised in ulcerative colitis (66-71).

Bacteria in Crohn's colitis. In Crohn's colitis, the number of mucosal bacteria such as *Mycobacterium avium paratuberculosis, C. difficile, Ruminococcus gnavus, Enterobacteriaceae* and *E. coli* are increased, while bacterial diversity, Clostridium groups IV, XIVa (*F. prausnitzii*) and Bifidobacteria and Lactobacillia, are reduced (72-77).

Lysozyme is up-regulated in microscopic colitis and in inflammatory bowel disease. In collagenous colitis lysozyme is up-regulated in the colonic crypts and in metaplastic Paneth cells (9). In lymphocytic colitis, lysozyme is up-regulated in *lamina propria* macrophages that underline the surface epithelium (9) as well as in the lower part of the crypts. The increased production of lysozyme in collagenous colitis and in lymphocytic colitis supports a bacterial aetiology for these two diseases. The different mucosal cell types displaying increased production of lysozyme (epithelial vs. macrophages) substantiates the notion that collagenous colitis and lymphocytic colitis might be two different maladies. Notably, collagenous colitis and lymphocytic colitis were also found in non-human primates having protracted intractable diarrheas (78).

In active ulcerative colitis, lysozyme is up-regulated in metaplastic Paneth cells (left colon) and in the deep half of the crypts. In ulcerative colitis in remission, lysozyme is up-regulated in metaplastic Paneth cells (9). No lysozyme expression is recorded in the crypts.

In Crohn's colitis lysozyme up-regulation is found in metaplastic Paneth cells (left colon), in the crypts as well as in the *lamin propria mucosae* (9). The increased lysozyme production in the colonic mucosa in patients with inflammatory bowel disease may highlight an amplified mucosal protection against the alien pathogenic bacteria proliferating in the colonic microenvironment in these patients (65-77).

In sum, bacterial antibiotic resistance continues to exhaust our supply of effective antibiotics. The future challenge is how to solve the increasing menace of bacterial resistance to antibacterial drugs. In his Presidential address, Alexander Fleming said 80 years ago: "I choose lysozyme as the subject for this address for two reasons, firstly because I have a fatherly interest in the name and, secondly, because its importance in connection with natural immunity does not seem to be generally appreciated" (3). Perhaps, the challenging legacy of Alexander Fleming together with the more recent bacterial resistance to commercial antibiotics are the explanation for the boost in research on the potential use of lysozyme for the treatment of infectious diseases. This research includes not only laboratory and farmed animals, but also agricultural products (78-88).

Conflicts of Interest

None.

References

1 Kaper JB and Sperandio V: Bacterial Cell-to-Cell Signaling in the Gastrointestinal Tract. Infect Immun *73*: 3197-3209, 2005,

2 Dominguez-Bello MG, Blaser MJ, Ley RE and Knight R: Development of the human gastrointestinal microbiota and insights from high-throughput sequencing. Gastroenterology *140*: 1713-1719, 2011.

3 Fleming A: On a remarkable bacteriolytic element found in tissues and secretions. Proc Roy Soc *93*: 306-312, 1922.

4 Yoshimura K, Toibana A and Nakahama K: Human lysozyme: sequencing of a cDNA, and expression and secretion by Saccharomyces cerevisiae. Biochem Biophys Res Commun *150*: 794-801, 1988.

5 Peters C, Kruse U, Pollwein R, Grzeschik K and Sippel T: The human lysozyme gene. Sequence organization and chromosomal localization. Eur J Bioch *182*: 507-512, 1989.

6 Sahoo NR, Kumar P, Bhusan B, Bhattacharya T, Dayal S and Sahoo M: Lysozyme in Livestock: A Guide to Selection for Disease Resistance: a Review J Anim Sci Adv *2*: 347-360, 2012.

7 Rubio CA and Lörinc E: Lysozyme is up-regulated in Barrett's mucosa. Histopathology *58*: 796-799, 2011.

8 Rubio CA and Befrits R: Increased lysozyme expression in gastric biopsies with intestinal metaplasia and pseudopyloric metaplasia. Int J Clin Exp Med *2*: 248-253. 2009.

9 Rubio CA: Lysozyme expression in microscopic colitis. J Clin Pathol *64*: 510-515, 2011,

10 Rubio CA: Lysozyme-rich mucus metaplasia in duodenal crypts supersedes Paneth cells in celiac disease. Virchows Arch *459*: 339-346. 2011.

11 Rubio CA. Lysozyme expression in microscopic colitis. J Clin Pathol *64*: 510-515, 2011.

12 Rubio CA: The Natural Antimicrobial Enzyme Lysozyme is Up-Regulated in Gastrointestinal Inflammatory Conditions. Pathogens *3*: 73-92, 2014.

13 Appelman HD, Umar A, Orlando RC, Sontag SJ, Nandurkar S, El-Zimaity H, Lanas A, Parise P, Lambert R and Shields HM: Barrett's esophagus: natural history. Ann NY Acad Sci 1232: 292-308, 2011.

14 Rubio CA, Dick EJ, Schlabritz-Loutsevitch NE, Orrego A and Hubbard GB: The columnar-lined mucosa at the gastroesophageal junction in non-human primates. Int J Clin Exp Pathol *2*: 481-488, 2009.
15 Rubio CA, Nilsson JR, Owston M and Dick EJ: The length of the Barrett's mucosa in baboons, revisited. Anticancer Res *32*: 3115-3118, 2012.
16 Spechler S and Goyal R: Barrett's esophagus. N Engl J Med *315*: 362-367, 1986.
17 Sampliner RE: Practice guidelines on the diagnosis, surveillance, and therapy of Barrett's esophagus. The Practice Parameters Committee of the American College of Gastroenterology. Am J Gastroenterol *93*: 1028-1032, 1998.
18 Playford RJ: New British Society of Gastroenterology (BSG) guidelines for the diagnosis and management of Barrett's oesophagus. Gut *55*: 442-444, 2006.
19 Fiocca R, Mastracci L, Milione M, Parente P and Savarino V: Gruppo Italiano Patologi Apparato Digerente (GIPAD) and Società Italiana di Anatomia Patologica e Citopatologia Diagnostica/International Academy of Pathology, Italian division (SIAPEC/IAP). Microscopic esophagitis and Barrett's esophagus: The histology report. Dig Liver Dis *43(Suppl 4)*: S319-S330, 2011.
20 Takubo K, Vieth M, Aida J, Sawabe M, Kumagai Y, Hoshihara Y and Arai T: Differences in the definitions used for esophageal and gastric diseases in different countries: Endoscopic definition of the esophagogastric junction, the precursor of Barrett's adenocarcinoma, the definition of Barrett's esophagus, and histologic criteria for mucosal adenocarcinoma or high-grade dysplasia. Digestion *80*: 248-257, 2009.
21 Waldum HL, Hauso Ø and Sandvik AK: PPI-induced hypergastrinaemia and Barrett's mucosa: The fog thickens. Gut *59*: 1157-1158, 2010.
22 Yang L, Lu X, Nossa CW, Francois F, Peek R and Pei Z: Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. Gastroenterology *137*: 588-597, 2009.
23 Pei Z, Yang L, Peek R M Jr., Levine S M, Pride D T and Blaser M J: Bacterial biota in reflux esophagitis and Barrett's esophagus. World J Gastroent *11*: 7277-7283, 2005.
24 Macfarlane S, Furrie E, Macfarlane G and Dillon J: Microbial colonization of the upper gastrointestinal tract in patients with Barrett's esophagus. Clin Infect Dis *45*: 29-38, 2007.
25 Yang L, Lu X, Nossa CW, Francois F and Pei Z: Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. Gastroenterology *137*: 588-597, 2009.
26 Liu N, Ando T, Ishiguro K, Maeda O, Watanabe O, Funasaka K, Nakamura M, Miyahara R, Ohmiya N and Goto H: Characterization of bacterial biota in the distal esophagus of Japanese patients with reflux esophagitis and Barrett's esophagus. BMC Infect Dis *13*: 130-136, 2013.
27 Rubio CA: Lysozyme is up-regulated in columnar-lined Barrett's mucosa: a possible natural defence mechanism against Barrett's esophagus-associated pathogenic bacteria. Anticancer Res *32*: 5115-5119, 2012.
28 Sarosi G, Brown G, Jaiswal K, Feagins LA, Lee E, Crook T, Souza RF, Zou YS, Shay JW and Spechler SJ: Bone marrow progenitor cells contribute to esophageal regeneration and metaplasia in a rat model of Barrett's esophagus. Dis Esophagus *21*: 43-50, 2008.
29 Rubio CA: Putative Stem Cells in Mucosas of the Esophago-Gastrointestinal Tract. Chapter 10. *In*: Stem Cell, Regenerative Medicine and Cancer Ed. Shree Ram Singh, Nova Science Publishers, Inc. Haupauge, NY, USA. pp. 281-310. 2011.
30 Misiewicz J, Tygat G and Goodwin C: The Sydney System: a new classification of gastritis. Working Party Reports *1*: 10-11. 1990.
31 Warren J and Marshall B: Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet i:, 1273-1275, 1983.
32 Oh JD, Kling-Backhed H, Giannakis M, Engstrand LG and Gordon JI: Interactions between gastric epithelial stem cells and *Helicobacter pylori* in the setting of chronic atrophic gastritis. Curr Opin Microbiol *9*: 21-27, 2006.
33 Correa P: A human model of gastric carcinogenesis. Cancer Res *48*: 1319-1326, 1988.
34 Giannella R, Broitman S and Zamcheck N: Gastric acid barrier to ingested micro-organisms in man: studies in vivo and *in vitro*. Gut *13*: 251-256, 1972.
35 Rubio CA, Jaramillo E, Suzuki G, Lagergren P, Nesi G: Antralization of the gastric mucosa of the incisura angularis an its gastrin expression. Int J Clin Exp Pathol *2*: 65-70, 2009.
36 Nakajima S, Nishiyama Y, Yamaoka M, Yasuoka T and Cho E: Changes in the prevalence of *Helicobacter pylori* infection and gastrointestinal diseases in the past 17 years. J Gastroenter Hepatol Suppl *25*: S99-S110, 2010.
37 Campbell D, Warren B, Thomas J, Figura N, Telford J. and Sullivan P: The African enigma: Low prevalence of gastric atrophy, high prevalence of chronic inflammation in West African adults and children. Helicobacter *6*: 263-267, 2001.
38 Graham DY, Lu H and Yamaoka Y: African, Asian or Indian enigma, the East Asian *Helicobacter pylori*: facts or medical myths. J Dig Dis *10*: 77-84, 2009.
39 Saieva C, Rubio CA, Nesi, G, Zini E and Filomena A: Classification of gastritis in first-degree relatives of patients with gastric cancer in a high cancer-risk area in Italy. Anticancer Res *32*: 1711-1716, 2012.
40 Torbenson M, Abraham SC, Boitnott J, Yardley JH and Wu T: Autoimmune gastritis: distinct histological and immuno-histochemical findings before complete loss of oxyntic glands. Mod Pathol *15*: 102-109. 2002.
41 Petersson F, Borch K and Franzén E: Prevalence of subtypes of intestinal metaplasia in the general population and in patients with autoimmune chronic atrophic gastritis. Scand J Gastroenterol *37*: 262-266, 2002.
42 Guerre J, Vedel G, Gaudric M, Paul G and Cornuau J: Bacterial flora in gastric juice taken at endoscopy in 93 normal subjects. Pathol Biol *34*: 57-60. 1986
43 Monstein HJ, Tiveljung A, Kraft CH, Borch K and Jonasson J: Profiling of bacterial flora in gastric biopsies from patients with *Helicobacter pylori* associated gastritis and histologically normal control individuals by temperature gradient gel electrophoresis and 16S rDNA sequence analysis. J Med Microbiol *49*: 817-822, 2000.
44 Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, Perez-Perez G, Blaser MJ and Relman DA: Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci *103*: 732-7372006,
45 Li XX, Wong GL, To KF, Wong VW, Lai LH, Chow DK, Lau JY, Sung JJ, and Ding C: Bacterial Microbiota Profiling in Gastritis without *Helicobacter pylori* Infection or Non-Steroidal Anti-Inflammatory Drug Use. PLoS ONE *4*: e7985, 2009.

46 Shand AG, Taylor AC, Banerjee M, Lessels A, Coia J, Clark C, Haites N and Ghosh S: Gastric fundic gland polyps in south-east Scotland: absence of adenomatous polyposis coli gene mutations and a strikingly low prevalence of *Helicobacter pylori* infection. J Gastroenterol Hepatol *17*: 1161-1164, 2002.
47 Rubio CA: Plugs clog the glandular outlets in fundic gland polyps. Int J Clin Exp Pathol *3*: 69-74, 2009.
48 Rubio CA: My approach to reporting a gastric biopsy. J Clin Pathol *60*: 160-166, 2007.
49 Rubio CA: Lysozyme overexpression in fundic gland polyps. Anticancer Res *30*: 1021-1024, 2010.
50 Shousha S, el-Sherif AM, el-Guneid A and Arnaout AH: *Helicobacter pylori* and intestinal metaplasia: comparison between British and Yemeni patients. Am J Gastroenterol *88*: 1373-1376, 1993.
51 Rubio CA, Kato Y, Sugano H and Kitagawa T: Intestinal metaplasia of the stomach in Swedish and Japanese patients without ulcers or carcinoma. Jpn J Cancer Res *78*: 467-472, 1987.
52 Rubio CA, Jessurum J and Kato Y: Low frequency of intestinal metaplasia in gastric biopsies from Mexican patients: a comparison with Japanese and Swedish patients. Jpn J Cancer Res *83*: 491-494, 1992.
53 Dewar DH and Ciclitira PJ: Clinical features and diagnosis of celiac disease. Gastroenterology *128(Suppl 1)*: S19-24, 2005.
54 Ivarsson A, Högberg L and Stenhammar L: Swedish Childhood Coeliac Disease Working Group. The Swedish Childhood Coeliac Disease Working Group after 20 years: history and future. Acta Paediatr *99*: 1429-1431, 2010.
55 Ivarsson A, Myléus A, Norström F, van der Pals M, Rosén A, Högberg L, Danielsson L, Halvarsson B, Hammarroth S, Hernell O, Karlsson E, Stenhammar L, Webb C, Sandström O and Carlsson A: Celiac disease revealed in 3% of Swedish 12-year-olds born during an epidemic. J Pediatr Gastroenterol Nutr *49*: 170-176, 2009.
56 Sánchez E, Donat E, Ribes-Koninckx C, Calabuig M and Sanz Y: Intestinal Bacteroides species associated with coeliac disease. J Clin Pathol *63*: 1105-1111, 2010.
57 Schippa S, Iebba V, Barbato M, Di Nardo G, Totino V, Checchi MP, Longhi C, Maiella G, Cucchiara S and Conte MP: A distinctive 'microbial signature' in celiac pediatric patients BMC Microbiol *10*: 175-179, 2010.
58 Forsberg G, Fahlgren A, Hörstedt P, Hammarström S, Hernell O and Hammarström M: Presence of bacteria and innate immunity of intestinal epithelium in childhood celiac disease. Am J Gastroenterol *99*: 894-904, 2004.
59 Ou G, Hedberg M, Hörstedt P, Baranov V, Forsberg G, Drobni M, Sandström O, Wai SN, Johansson I, Hammarström ML, Hernell O and Hammarström S: Proximal small intestinal microbiota and identification of rod-shaped bacteria associated with childhood celiac disease. Am J Gastroenterol *104*: 3058-3067, 2009.
60 Rubio CA: Signaling pathways, gene regulation and duodenal neoplasias. Chapter 6. *In*: Signaling, gene regulation and cancer. Singh SR (ed). Nova Science Publishers, Inc. Haupauge, NY, USA, pp. 83-110, 2013.
61 Lindström CG: "Collagenous colitis" with watery diarrhoea: a new entity? Pathol Eur *11*: 87-89, 1976.
62 Giardiello FM, Lazenby AJ, Bayless TM, Levine EJ, Bias WB, Ladenson PW, Hutcheon DF, Derevjanik NL and Yardley JH: Lymphocytic (microscopic) colitis. Clinico-pathologic study of 18 patients and comparison to collagenous colitis. Dig Dis Sci *34*: 1730-1738, 1989.
63 Wickbom A, Bohr J, Eriksson S, Udumyan R, Nyhlin N and Tysk C: Stable Incidence of Collagenous Colitis and Lymphocytic Colitis in Örebro, Sweden, 1999-2008: A Continuous Epidemiologic Study. Inflamm Bowel Dis *19*: 2387-2393, 2013.
64 Gustafsson RJ, Ohlsson B, Benoni C, Jeppsson B, and Olsson C: Mucosa-associated bacteria in two middle-aged women diagnosed with collagenous colitis. World J Gastroenterol *18*: 1628-1634, 2012.
65 Helal T E, Ahmed NS and El Fotoh OA: Lymphocytic colitis: a clue to bacterial etiology. World J Gastroenterol *11*: 7266-7271, 2005.
66 Khalil NA, Walton GE, Gibson GR, Tuohy KM and Andrews SC: *In vitro* batch cultures of gut microbiota from healthy and ulcerative colitis (UC) subjects suggest that sulphate-reducing bacteria levels are raised in UC and by a protein-rich diet. Int J Food Sci Nutr *65*: 79-88, 2014.
67 Kumari R, Ahuja V and Paul J: Colonisation by Faecalibacterium prausnitzii and maintenance of clinical remission in patients with ulcerative colitis. World J Gastroenterol *19*: 3404-3408, 2013.
68 Varela E, Manichanh C, Gallart M, Torrejón A, Borruel N, Casellas F, Guarner F and Antolin M: Colonisation by Faecalibacterium prausnitzii and maintenance of clinical remission in patients with ulcerative colitis. Aliment Pharmacol Ther *38*: 151-156, 2013.
69 Pilarczyk-Zurek M, Chmielarczyk A, Gosiewski T, Tomusiak A, Adamski P, Zwolinska-Wcislo M, Mach T, Heczko PB and Strus M: Possible role of *Escherichia coli* in propagation and perpetuation of chronic inflammation in ulcerative colitis. BMC Gastroenterol *13*: 61-65, 2013.
70 Yukawa T, Ohkusa T, Shibuya T, Tsukinaga S, Mitobe J, Takakura K, Takahara A, Odahara S, Matsudaira H, Nagatsuma K, Kitahara T, Kajihara M, Uchiyama K, Arakawa H, Koido S, and Tajiri H: Nested culture method improves detection of Fusobacterium from stool in patients with ulcerative colitis. Jpn J Infect Dis *66*: 109-111, 2013.
71 Kabeerdoss J, Sankaran V, Pugazhendhi S and Ramakrishna BS: Clostridium leptum group bacteria abundance and diversity in the fecal microbiota of patients with inflammatory bowel disease: a case-control study in India. BMC Gastroenterol *13*: 20-26: 2013.
72 Gałecka M, Szachta P, Bartnicka A, Łykowska-Szuber L, Eder P and Schwiertz A: Faecalibacterium prausnitzii and Crohn's disease - is there any connection? Pol J Microbiol *62*: 91-95: 2013.
73 Kale-Pradhan PB, Zhao JJ, Palmer JR and Wilhelm SM: The role of antimicrobials in Crohn's disease. Expert Rev Gastroenterol Hepatol *7*: 281-288, 2013.
74 Nickerson K and McDonald C: Crohn's disease-associated adherent-invasive *Escherichia coli* adhesion is enhanced by exposure to the ubiquitous dietary polysaccharide maltodextrin. PLoS One *7*: e52132, 2012.
75 Erickson AR, Cantarel BL, Lamendella R, Darzi Y, Mongodin EF, Pan C, Shah M, Halfvarson J, Tysk C, Henrissat B, Raes J, Verberkmoes NC, Fraser CM, Hettich RL and Jansson JK: Integrate metagenomics/metaproteomics reveals human host-microbiota signatures of Crohn's disease. PLoS One *7*: e49138, 2012.

76 Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, Essers J, Mitrovic M, Ning K, Cleynen I, Theatre E, Spain SL, Raychaudhuri S, Goyette P, Wei Z, Abraham C, Achkar JP, Ahmad T, Amininejad L, Ananthakrishnan AN, Andersen V, Andrews JM, Baidoo L, Balschun T, Bampton PA, Bitton A, Boucher G, Brand S, Büning C, Cohain A, Cichon S, D'Amato M, De Jong D, Devaney KL, Dubinsky M, Edwards C, Ellinghaus D, Ferguson LR, Franchimont D, Fransen K, Gearry R, Georges M, Gieger C, Glas J, Haritunians T, Hart A, Hawkey C, Hedl M, Hu X, Karlsen TH, Kupcinskas L, Kugathasan S, Latiano A, Laukens D, Lawrance IC, Lees CW, Louis E, Mahy G, Mansfield J, Morgan AR, Mowat C, Newman W, Palmieri O, Ponsioen CY, Potocnik U, Prescott NJ, Regueiro M, Rotter JI, Russell RK, Sanderson JD, Sans M, Satsangi J, Schreiber S, Simms LA, Sventoraityte J, Targan SR, Taylor KD, Tremelling M, Verspaget HW, De Vos M, Wijmenga C, Wilson DC, Winkelmann J, Xavier RJ, Zeissig S, Zhang B, Zhang CK, Zhao H; International IBD Genetics Consortium (IIBDGC), Silverberg MS, Annese V, Hakonarson H, Brant SR, Radford-Smith G, Mathew CG, Rioux JD, Schadt EE, Daly MJ, Franke A, Parkes M, Vermeire S, Barrett JC, and Cho JH: Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature *491*: 119-124, 2012.

77 Shanahan F: The microbiota in inflammatory bowel disease: friend, bystander, and sometime-villain. Nutr Rev *70(Suppl 1)*: S31-S37, 2012.

78 Rubio CA and Hubbard GB: Chronic colitis in baboons: similarities with chronic colitis in humans. In Vivo *15*: 109-116, 2001.

79 Muriel-Galet V, Talbert JN, Hernandez-Munoz P, Gavara R and Goddard JM: Covalent Immobilization of Lysozyme on Ethylene Vinyl Alcohol Films for Nonmigrating Antimicrobial Packaging Applications. J Agric Food Chem *61*: 6720-6727, 2013.

80 Ibrahim HR, Imazato K and Ono H: Human lysozyme possesses novel antimicrobial peptides within its N-terminal domain that target bacterial respiration. J Agric Food Chem *59*: 10336-10345, 2011.

81 Oliver WT and Wells JE: Lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology in nursery pigs. J Anim Sci *91*: 3129-3136, 2013.

82 Teneback C, Scanlon T, Wargo M, Bement J, Griswold K and Leclair LW: Bioengineered lysozyme reduces bacterial burden and inflammation in a murine model of mucoid Pseudomonas aeruginosa lung infection. Antimicrob Agents Chemother *57*: 5559-5564, 2013.

83 Pridgeon JW, Klesius PH, Dominowski PJ, Yancey PJ and Kievit S: Chicken-type lysozyme in channel catfish: Expression analysis, lysozyme activity, and efficacy as immunostimulant against Aeromonas hydrophila infection Fish Shellfish Immunol *35*: 680-688, 2013.

84 Cegielska-Radziejewska R and Szablewski T: Effect of modified lysozyme on the microflora and sensory attributes of ground pork. J Food Prot *76*: 338-342, 2013.

85 Lamppa JW, Tanyos and Griswold KE: Engineering *Escherichia coli* for soluble expression and single step purification of active human lysozyme. J Biotechnol *164*: 1-8, 2013.

86 Rosu V, Bandino E and Cossu A: Unraveling the transcriptional regulatory networks associated to mycobacterial cell wall defective form induction by glycine and lysozyme treatment. Microbiol Res *168*: 153-164, 2013.

87 Scanlon TC, Teneback CC, Gill A, Bement JL, Weiner JA, Lamppa JW, Leclair LW and Griswold KE: Enhanced antimicrobial activity of engineered human lysozyme. ACS Chem Biol *5*: 809-818, 2010.

88 Bhavsar T, Liu M, Hardej D, Liu X and Cantor J: Aerosolized recombinant human lysozyme ameliorates Pseudomonas aeruginosa-induced pneumonia in hamsters. ACS Chem Biol *5*: 809-818, 2010.

Received August 31, 2015
Revised September 30, 2015
Accepted October 19, 2015