BIOTASCOPE

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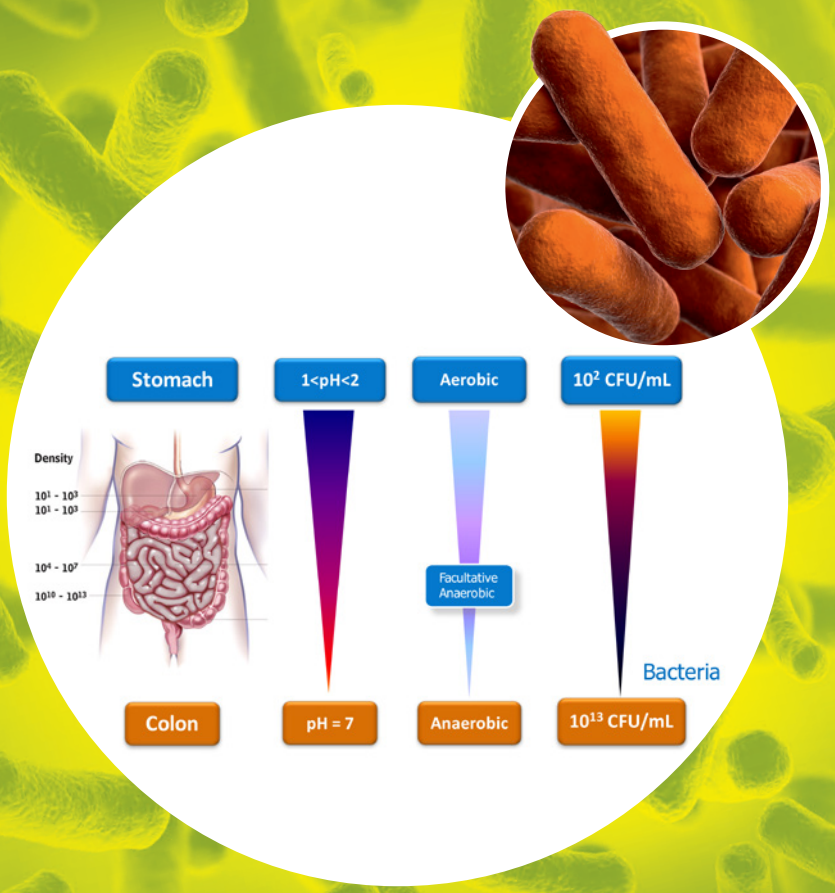
Translational Science in Microbiota



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Contents

Editorial

Dear Colleagues,

BIOTASCOPE as we know it has come to an end. This journal will continue to live, but in a different format. To date, BIOTASCOPE has included review articles from well-known world-wide authors, highlights of the best studies from major international meetings, and summaries of recent articles in the field of microbiota. Articles were divided according to the specific needs of our readers, including pediatric or internal medicine literature and basic translational clinical science.

This issue is particularly important. The microbiome of the esophagus, an undermined but interesting topic, is reviewed by Miguel Valdovinos from Mexico. The relationship between microbiota and GERD, Barrett's esophagus as well as esophageal adenocancer are also discussed. Currently, many articles on the complications of proton pump inhibitors, which are one of the most widely used medications in the world, are being published. Carmelo Scarpignato, from Italy, has written a clinical review article that focuses on this very important issue: the effect of proton pump inhibitors on gut microbiota. He does not, however, limit his analysis to bacterial overgrowth, but also adopts a broader approach, discussing specific microbiota in different parts of the gastrointestinal tract and the effect of acid suppression. Our readers who are interested in pediatric age groups will appreciate the review article by Pearay L. Ogra from New York (USA), which focuses on the development of the mucosal immune system. Firstly, it gives an overview of skin development and mucosal microbiome, with an emphasis on defense mechanisms, immune responses and the role of the microbiome; secondly, it highlights the role of the microbiome in different clinical settings and pathologic states.

We are grateful to Tarkan Karakan from Turkey, who contributed to most of the 'essence from the literature' items of this issue and summarized four fascinating articles. In the first article, the effects of a FODMAP diet on pediatric age patients with irritable bowel syndrome were evaluated. This is the first study investigating both this type of diet and the composition of gut microbiota. The following two summaries were of articles chosen for complementing other articles in this issue. One, published in PLoSOne, was similar to the review article by Valdovinos in that it focused on eosinophilic esophagitis and esophageal microbiota. The other study, published in Gastroenterology by Freedberg et al, showed the effect of proton pump inhibitors on some bacterial taxa and their gene expression, resulting with a predisposition to C. difficile. The last summary is that of a study conducted by Vicram et al which suggests that there is a "gut-vascular axis" which controls vascular endothelial function via the microbiome.

The importance of microbiota is increasing tremendously and gaining a more important place in major international meetings. Prof. Andras Arato from Budapest (Hungary) kindly gave a summary of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Annual Meeting which was organized in Athens (Greece). Dimitry Bordin from Russia attended the Digestive Diseases Week in San Diego (USA) and you can read the latest developments in the field in his summary.

«All GOOD things must come to an end
to make way for BETTER things to happen
because the BEST is yet to come»…

Best wishes from the International Study Group of Probiotics (ISGoP).

Sincerely,

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MICROBIOME OF THE ESOPHAGUS IN HEALTH AND DISEASE

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INTRODUCTION

A great variety of microbial communities (microbiota) and their genes (microbiome) exist throughout the human body with fundamental functions in health and relevant roles in disease. Microbes of the human body include bacteria, viruses, fungi, and archaea. Bacteria, the most studied of the microorganisms, can reach counts 10 times higher than those of the cells of the human body and have 150 times more genes than the human genome[1]. The bacterial composition of the human gut microbiota has been the subject of intense research over the past decade. We now know that the gut microorganisms and their metabolites play a very important role in nutrition and energy metabolism[2], immune functions[3], protection against pathogen invasion, and other important physiologic activities[4]. On the other hand, dysbiosis, or alteration of the microbial composition of the gut microbiota, has been associated with different gastrointestinal diseases, such as inflammatory bowel disease[5], irritable bowel syndrome[6], *Clostridium difficile* infection[7], and gastrointestinal (GI) cancers[8].

Recent studies have shown that the esophagus also has a complex microbiota. Approximately 140 bacterial species in the normal distal esophagus have been described[9; 10]. Interestingly, different analyses have shown that the esophageal microbiome of patients with gastroesophageal reflux disease (GERD), Barrett's esophagus (BE), esophageal adenocarcinoma (EAC)[11; 12] and eosinophilic esophagitis (EoE)[13] differs from the microbiome of normal subjects. These findings suggest that dysbiosis could play a role in inflammation and carcinogenesis of the esophagus.

MICROBIOME OF THE NORMAL ESOPHAGUS

Initial studies using luminal washes, brush samples, and biopsies of the esophagus suggest that the normal esophagus could have transitory microorganisms coming from the oropharynx by swallowing or from the stomach through gastroesophageal reflux[9; 14]. In these studies, *Streptococcus viridans* was found to be the most numerous bacteria in the esophagus and oropharynx of normal subjects. Other common bacteria found in the esophagus were *Neisseria spp., Haemophilus spp.,* and *Prevotella spp.* These findings suggest that the human esophagus could be colonized with a resident flora of its own, even though it has similarities with the microbiota present in the oral cavity.

The most recent studies carried out with molecular techniques for characterizing the esophageal microbiota have shown that the large majority of the esophageal bacteria were known and cultivable. Pei and colleagues[10] used broad-range 16S rDNA PCR and found that members of six phyla, Firmicutes, Bacteroides, Actinobacteria, Proteobacteria, Fusobacteria, and TM7, were represented in the normal esophagus of adult subjects. *Streptococcus, Prevotella, and Veillonellance were the most prevalent genera in esophageal biopsies.* Fillon and colleagues[15] characterized the esophageal microbiome in children with normal esophageal mucosa using a novel device, a capsule-based string technology called the Enterotest™ (EST), and found that microbiota phylum-level diversity was similar to esophageal biopsies and EST. *Streptococcus, Prevotella, and Veillonellance* were the predominant genera in esophageal samples, a finding that was consistent with the previous study.

MICROBIOME OF THE ESOPHAGUS IN GERD, BARRETT'S ESOPHAGUS AND ADENOCARCINOMA

Several studies have analyzed and compared the microbial composition of the esophageal microbiota of normal subjects with the microbiota of patients with GERD, BE, and EAC.

In their study, Yang and colleagues[16] analyzed the diversity of the microbiota in biopsies from the distal esophagus in subjects with a normal esophagus and in patients with esophagitis and BE, using 16S rDNA sequencing.

They found that the esophageal microbiome can be classified into two distinct clusters or two microbiome types. None of the two types of clusters correlated exclusively with GERD phenotypes. The type I microbiome had a greater association with normal esophagus, whereas the type II microbiome was associated with GERD phenotypes, including reflux esophagitis and BE. The type I microbiome was dominated by Gram-positive bacteria. The type II microbiome was composed of Gram-negative bacteria including Bacteroidetes, Proteobacteria, Fusobacteria, and Spirochaetes. The relative abundance of Streptococcus, the dominant genus in the esophageal microbiome, was significantly higher in the type I microbiome (78.8%) than in the type II microbiome (30%). In the type II microbiome, the increase in the relative abundance of Gram-negative anaerobes or microaerophiles compensated for the decrease in the relative abundance of *Streptococcus*. The predominant genera were Veillonella, Prevotella, Haemophilus, Neisseria, Rothia, Granulicatella, Campylobacter, Porphyromonas, Fusobacterium, and Actinomyces. Gram-negative bacteria comprised 53.4% of the type II microbiome, but only 14.9% of the type I microbiome.
Liu and colleagues[12] found that the total amount of bacterial DNA extracted from esophageal biopsies did not significantly differ among normal subjects, patients with reflux esophagitis, and BE. However, a phylum-level analysis showed that esophageal bacterial composition differed between these groups. Each group had a different number of phyla: four phyla in patients with normal esophagus, six in those with reflux esophagitis, and five in those patients with BE. Phyla composition was different among these patients. Fusobacteria were found in patients with reflux esophagitis or BE, but not in normal esophagus.

Blackett and colleagues[11] identified esophageal microbiota by culture analysis and molecular techniques in esophageal biopsies of 4 groups of patients: subjects with normal esophagus, patients with reflux esophagitis, BE, or EAC. They isolated 111 species belonging to 26 bacterial genera and found that there was a significant reduction in the bacterial counts of patients with reflux esophagitis and BE for all the genera, except Campylobacter. The dominant species was *Campylobacter concisus*. These findings were not observed in normal subjects or in those with EAC. It was interesting that the molecular analysis of cytokine expression in esophageal biopsies showed no statistical differences between GERD, EAC patients and controls for pro-inflammatory cytokines. However, there was a significant increase in IL-18 expression in those patients colonized by Campylobacter, compared with the non-colonized patients.

In summary, these three studies reveal important findings: 1) the esophageal microbiome of patients with reflux esophagitis, BE, and EAC is different from that of the subjects with normal esophagus; 2) a shift in the microbiome from Gram-positive relative abundance to Gram-negative relative abundance in the distal esophagus is probably associated with GERD phenotypes and disease progression; 3) some bacterial species that colonize the esophagus of GERD, BE and EAC patients can induce proinflammatory interleukin expression. However, the effect of age, sex, diet, proton pump inhibitor (PPI) use, esophageal motility disorders, and other possible confounding factors in relation to the esophageal microbiota still needs to be determined.

MICROBIOME OF THE ESOPHAGUS IN EOSINOPHILIC ESOPHAGITIS

There are very few studies on the esophageal microbiome in patients with EoE. Harris and colleagues[13] analyzed the bacterial load and bacterial communities in secretions of the esophageal mucosa using quantitative PCR and 16S rRNA gene amplification and pyrosequencing in children and adults with untreated and treated EoE and GERD, and in normal mucosa using EST. The relevant findings of this study were: 1) the bacterial load detected in all patients with EoE was significantly higher than in normal subjects and these results were not influenced by treatment or disease activity; 2) the bacterial load identified in GERD patients was also significantly increased compared with that of normal subjects; 3) Haemophilus was the dominant genus in untreated EoE patients compared with normal subjects; and 4) Streptococcus was decreased in GERD patients on PPI treatment compared with controls. In addition, Benitez and colleagues[17] characterized the oral and esophageal microbiome of children with EoE and non-EoE controls. EoE patients were also studied longitudinally before and after food elimination and diet reintroduction of allergenic food. Microbial composition was determined in oral swabs and esophageal biopsies using 16S rRNA gene sequencing. This study showed that proportions of esophageal bacterial communities were significantly different in EoE patients compared with non-EoE controls. EoE esophageal microbiota was enriched by Proteobacteria, including Neisseria and Corynebacterium. In contrast, Firmicutes were dominant in non-EoE controls. No significant differences were detected between inactive EoE samples and non-EoE controls. Food elimination did not lead to significant differences in either oral or esophageal microbiota of EoE patients, whereas diet reintroduction of allergenic foods resulted in the predominance of Ganulicatella and Campylobacter genera in the esophagus.

In short, these initial studies suggest that the esophageal microbiome of the patients with EoE is different from that of normal subjects. It is possible that the inflammatory activity of the disease, more than the EoE itself, is the determining factor in the microbial composition of the esophageal microbiota in this group of patients. Further studies are required to determine the influence of dietary interventions on the esophageal microbiome of patients with EoE.

POSSIBLE ROLE OF DYSBIOSIS IN THE PATHOGENESIS OF GERD, BARRETT'S ESOPHAGUS, AND ADENOCARCINOMA

The pathophysiology of GERD is complex and several mechanisms have been proposed to explain it. They include transient esophageal lower esophageal sphincter relaxations, esophageal dysmotility, delayed gastric emptying, loss of the antireflux barrier, and reduction of epithelial resistance[18]. BE is a complication of chronic gastroesophageal reflux. BE is defined as the presence of metaplastic columnar epithelium in the esophagus and predisposes to EAC. The risk for developing EAC in patients with BE has been estimated at 0.12% to 0.4% per patient-year[19]. In addition to chronic gastroesophageal reflux, other environmental factors such as smoking, obesity, and low consumption of fruit and vegetables have been associated with EAC[20]. Recent studies have suggested a possible role of esophageal microbiome alterations in the pathophysiology of GERD, BE, and EAC.

LPS/TLR/ NF-κB PATHWAY

The predominance of Gram-negative bacteria in the microbial composition of the esophageal microbiota recently identified in GERD-spectrum disorders has suggested possible hypotheses for explaining the pathogenesis of these esophageal conditions.

The cell wall components of Gram-negative bacteria, such as the lipopolysaccharides (LPS), flagellin, and lipopeptides can induce an inflammatory response through the activation of the Toll-like receptors (TLRs) that induce the activation of the nuclear factor kappa B (NF-kB) at the nuclear level for the production of different proinflammatory cytokines, such as tumor necrosis factor alpha, IL-1, and IL6.

Several studies have shown TLR expression in the esophageal mucosa in patients with reflux esophagitis, BE, and EAC, particularly TLR3, TLR4, TLR5, and TLR9[21; 22]. NF-κB activation up-regulates the expression of genes involved in inflammation, innate and adaptive immune responses, apoptosis inhibition, cell proliferation, and cell differentiation including IL-1β and IL-8[23] and cycloxygenase-2 (COX 2)[24]. Some studies have found that there is a gradient in the IL-1β and IL-8 levels that progressively increases in patients with reflux esophagitis, BE, and EAC[23]. Other studies have found that the COX-2 protein is expressed in Barrett's metaplasia and its level of expression is elevated in EAC[25]. An increase in inducible nitric oxide synthase (iNOS) expression in EAC has also been observed[26]. With these findings, Yang and colleagues[27; 28] have proposed that the interaction of the host with an altered esophageal microbiome can favor the inflammatory phenomena that occur in GERD-spectrum disorders and contribute to carcinogenesis. These effects could be explained by activation of the LPS/TLR4/ NF-κB pathway.

LIPOPOLYSACCHARIDES AND GASTROINTESTINAL MOTILITY

Some effects of LPS in gastrointestinal motor function have been reported in experimental animal models of endotoxemia, including slow gastric emptying and acceleration of intestinal transit[29]. Calatayud and colleagues[30] showed that an intraperitoneal injection of endotoxin in normal mice significantly delayed gastric emptying of a solid nutrient meal. This delay in gastric emptying induced by endotoxin can be prevented by indomethacin, a selective COX-2 inhibitor. Furthermore, Fan and colleague[31] have shown that endotoxin LPS causes a dose-dependent reduction in lower esophageal sphincter (LES) basal tone in opossums. These findings allow to speculate that an esophageal microbiome with a predominance of Gram-negative bacteria and its products, such as LPS, could reduce basal LES pressure and delay gastric emptying, thus favoring the development of gastroesophageal reflux[28]. Further research in humans is required to determine whether esophageal dysbiosis can cause LES incompetence, alter gastric emptying, and favor gastroesophageal reflux through mediators such as LPS.

THERAPEUTIC IMPLICATIONS

Dysbiosis identified in esophageal disorders consisting of a relative abundance of Gram-negative bacteria and the activation of the LPS/TLR4/NF-κB pathway could be a therapeutic intervention target, as suggested by Yang et al[28]. One treatment option would be the use of probiotics or antibiotics to revert the change in the esophageal microbiota to a predominance of Gram-positive bacteria with the potential reduction of the inflammatory phenomena and esophageal carcinogenesis. Another therapeutic approach would be to use NF-κB, COX-2, and iNOS-inhibiting drugs or compounds. Several experimental studies have shown that these inhibitors can have potential therapeutic effects on GERD-spectrum disorders. Curcumin, a NF-κB inhibitor and L-canavanine, a selective iNOS inhibitor, have been shown to increase apoptosis and chemosensitivity in EAC cell lines and block LPS-induced LES relaxation in mice, respectively[32; 33]. COX-2 inhibitors or nonsteroidal anti-inflammatory drugs reduce inflammation and may decrease the risk of progression from BE to EAC[34]. Further experimental studies and studies in humans are needed to determine the therapeutic potential of these agents.

CONCLUSIONS

There is new evidence that, similarly to other organs of the human body, such as the skin, vagina, intestine, and oral cavity, the normal esophagus has a complex microbiome. The esophageal microbiome of patients with GERD, BE, EAC, and EoE is different from that of normal subjects. The predominance of Gram-negative bacteria in the microbiome of patients with GERD-spectrum disorders appears to play a role in the pathogenesis of the inflammation in reflux esophagitis and BE and possibly in the development of EAC through the activation of the LPS/TLR4/ NF-κB pathway. These findings suggest that the use of probiotics and antibiotics, as well as the NF-κB, COX-2, and iNOS inhibitors, could have a therapeutic effect and modify esophageal dysbiosis and inflammation and, in turn, carcinogenesis.

Further studies are warranted in order to determine whether changes in the esophageal microbiome are responsible for initiating and promoting disease progression or if the presence and persistence of reflux induces the changes in the microbiome of the host.

References

(1) Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010; 464: 59-65.

(2) Flint HJ, Scott KP, Louis P, et al. The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol 2012; 9: 577-589.

(3) Palm NW, de Zoete MR, Flavell RA. Immune-microbiota interactions in health and disease. Clin Immunol 2015; 159: 122-127.

(4) Krishnan S, Alden N, Lee K. Pathways and functions of gut microbiota metabolism impacting host physiology. Curr Opin Biotechnol 2015; 36: 137-145.

(5) Matsuoka K, Kanai T. The gut microbiota and inflammatory bowel disease. Semin Immunopathol 2015; 37: 47-55.

(6) Ohman L, Simren M. Intestinal microbiota and its role in irritable bowel syndrome (IBS). Curr Gastroenterol Rep 2013; 15: 323.

(7) Wilcox MH. Clostridium difficile Infection. Infect Dis Clin North Am 2015; 29: i.

(8) Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer 2013; 13: 800-812.

(9) Gagliardi D, Makihara S, Corsi PR, et al. Microbial flora of the normal esophagus. Dis Esophagus 1998; 11: 248-250.

(10) Pei Z, Bini EJ, Yang L, et al. Bacterial biota in the human distal esophagus. Proc Natl Acad Sci U S A 2004; 101: 4250-4255.

(11) Blackett KL, Siddhi SS, Cleary S, et al. Oesophageal bacterial biofilm changes in gastro-oesophageal reflux disease, Barrett's and oesophageal carcinoma: association or causality? Aliment Pharmacol Ther 2013; 37: 1084-1092.

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

(13) Harris JK, Fang R, Wagner BD, et al. Esophageal microbiome in eosinophilic esophagitis. PLoS One 2015; 10: e0128346.

(14) Norder Grusell E, Dahlen G, Ruth M, et al. Bacterial flora of the human oral cavity, and the upper and lower esophagus. Dis Esophagus 2013; 26: 84-90.

(15) Fillon SA, Harris JK, Wagner BD, et al. Novel device to sample the esophageal microbiome--the esophageal string test. PLoS One 2012; 7: e42938.

(16) Yang L, Lu X, Nossa CW, et al. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. Gastroenterology 2009; 137: 588-597.

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

(18) Boeckxstaens GE, Rohof WO. Pathophysiology of gastroesophageal reflux disease. Gastroenterol Clin North Am 2014; 43: 15-25.

(19) Hvid-Jensen F, Pedersen L, Drewes AM, et al. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med 2011; 365: 1375-1383.

(20) Rubenstein JH, Shaheen NJ. Epidemiology, diagnosis, and management of esophageal adenocarcinoma. Gastroenterology 2015; 149: 302-317 e301.

(21) Sheyhidin I, Nabi G, Hasim A, et al. Overexpression of TLR3, TLR4, TLR7 and TLR9 in esophageal squamous cell carcinoma. World J Gastroenterol 2011; 17: 3745-3751.

(22) Mulder DJ, Lobo D, Mak N, et al. Expression of toll-like receptors 2 and 3 on esophageal epithelial cell lines and on eosinophils during esophagitis. Dig Dis Sci 2012; 57: 630-642.

(23) O'Riordan JM, Abdel-latif MM, Ravi N, et al. Proinflammatory cytokine and nuclear factor kappa-B expression along the inflammation-metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. Am J Gastroenterol 2005; 100: 1257-1264.

(24) Verbeek RE, Siersema PD, Ten Kate FJ, et al. Toll-like receptor 4 activation in Barrett's esophagus results in a strong increase in COX-2 expression. J Gastroenterol 2014; 49: 1121-1134.

(25) Shirvani VN, Ouatu-Lascar R, Kaur BS, et al. Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: Ex vivo induction by bile salts and acid exposure. Gastroenterology 2000; 118: 487-496.

(26) Wilson KT, Fu S, Ramanujam KS, et al. Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. Cancer Res 1998; 58: 2929-2934.

(27) Yang L, Chaudhary N, Baghdadi J, et al. Microbiome in reflux disorders and esophageal adenocarcinoma. Cancer J 2014; 20: 207-210.

(28) 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: 2138-2144.

(29) Cullen JJ, Caropreso DK, Ephgrave KS. Effect of endotoxin on canine gastrointestinal motility and transit. J Surg Res 1995; 58: 90-95.

(30) Calatayud S, Garcia-Zaragoza E, Hernandez C, et al. Downregulation of nNOS and synthesis of PGs associated with endotoxin-induced delay in gastric emptying. Am J Physiol Gastrointest Liver Physiol 2002; 283: G1360-1367.

(31) Fan YP, Chakder S, Gao F, et al. Inducible and neuronal nitric oxide synthase involvement in lipopolysaccharide-induced sphincteric dysfunction. Am J Physiol Gastrointest Liver Physiol 2001; 280: G32-42.

(32) Hartojo W, Silvers AL, Thomas DG, et al. Curcumin promotes apoptosis, increases chemosensitivity, and inhibits nuclear factor kappaB in esophageal adenocarcinoma. Transl Oncol 2010; 3: 99-108.

(33) Akaogi J, Barker T, Kuroda Y, et al. Role of non-protein amino acid L-canavanine in autoimmunity. Autoimmun Rev 2006; 5: 429-435.

(34) Kastelein F, Spaander MC, Biermann K, et al. Nonsteroidal anti-inflammatory drugs and statins have chemopreventative effects in patients with Barrett's esophagus. Gastroenterology 2011; 141: 2000-2008; quiz e2013-2004.

THE EFFECT OF PROTON PUMP INHIBITORS ON GUT MICROBIOTA

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ABSTRACT

Proton pump inhibitors (PPIs) are an effective class of drugs, widely prescribed in all age populations to reduce gastric acid production. The widespread, often lifelong, use of PPIs is of growing concern for the potential adverse effects resulting from such long-term therapy. While an effect of PPIs on the microbiota of the human gut is not unexpected, the consequences of long-term PPI therapy on the microbiota have only recently been elucidated, thanks to the use of omics tools to study the complex gut ecosystem. The mucosal-associated microbiota of the distal esophagus, which is altered in patients with esophagitis or Barrett's esophagus, is further modified by PPIs, leading to a significant increase in distal esophageal Lachnospiraceae, Comamonadaceae, and unclassified Clostridial families. Gastric acidity allows *Helicobacter pylori* to thrive and is also influenced by the presence of *H. pylori,* whose eradication relies on PPI-based eradication regimens. In the acid-depleted stomach there is a significant bacterial overgrowth, with a correlation between the pH values and cultured bacterial counts in gastric fluid. In the bowel, studies found a significant reduction in microbial diversity after (even short-term) use of PPIs. During PPI treatment significant changes in taxa associated with *Clostridium difficile* infection (increased Enterococcaceae and Streptococcaceae, decreased Clostridiales) and taxa associated with small intestine bacterial overgrowth (increased Micrococcaceae and Staphylococcaceae) were observed. As a consequence, long-term PPI users present a higher risk of *C. difficile* infection and small intestinal bacterial overgrowth compared with non-users. Taking into account the large spectrum of influence that gut microbiota has in health and disease, these side effects of acid suppression could have important consequences. However, it is worthwhile to emphasize that, based on the quality of the overall existing evidence, the benefits of PPI treatment outweigh the potential risks in the large majority of patients, especially if the PPI is prescribed in an appropriate indication. On the contrary, patients treated without appropriate therapeutic indication are only exposed to potential risks. Consequently, the overall focus should be on appropriateness of PPI therapy and on a regular assessment of the need for continued PPI treatment.

Key Words: acid suppression, adverse events, bacterial overgrowth, *Clostridium difficile* infection, gut, *Helicobacter pylori,* microbiota, NSAID-enteropathy, proton pump inhibitors.

INTRODUCTION

Proton pump inhibitors (PPIs) are the most potent inhibitors of gastric acid secretion available[1]. This class of antisecretory drugs has represented a real breakthrough in the treatment of acid-related diseases; their effectiveness span from peptic ulcers and eradication of *Helicobacter pylori,* to Zollinger-Ellison Syndrome, gastroesophageal reflux disease (GERD) and its complications, non-steroidal anti-inflammatory drug (NSAID)-associated gastro-duodenal ulcers as well as peptic ulcer bleeding[2].

All available PPIs[1] are benzimidazole derivatives and are most effective when the parietal cell is stimulated to secrete acid postprandially, a relationship that has important clinical implications for timing of administration. As the amount of H^+, K^+-ATPase present in the parietal cell is greatest after a prolonged fast, PPIs should be administered before the first meal of the day. In most individuals, once-daily dosing is sufficient to produce the desired level of acid inhibition, and a second dose, which is occasionally necessary, should be administered before the evening meal[1].
PPIs are an effective class of drugs, widely prescribed in all age populations. Health care providers are increasingly prescribing these medications for prolonged, sometimes lifelong, use and there is growing concern for potential adverse effects resulting from such long-term therapy[3]. In particular, inappropriate PPI

use is of great concern in the elderly, who often have multiple comorbidities, are receiving numerous medications, and therefore, are at an increased risk of long-term PPI-related adverse outcomes. Despite the potential of overuse and misuse to challenge the safety profile of PPIs, the tolerability of this drug class has been remarkably good. Adverse reactions generally occur at a rate of 1–3%, without no significant differences among PPIs. PPI-related adverse events involve the gastrointestinal (GI) tract as well as other organs and systems. The majority of these events have been discussed in comprehensive reviews[4, 5]; the aim of this paper is to summarize the current knowledge on the effect of PPIs on gut microbiota and their potential clinical consequences.

GUT MICROBIOTA IN HEALTH

Human microbiota is a complex living ecosystem consisting of unicellular microbes (mainly bacterial, but also archaeal *[Methanobrevibacter],* viral [i.e., bacteriophages] and eukaryotic [yeast]), which occupies almost every mucosal and cutaneous surfaces of our body. It has been estimated that microbes that stably live in human body amount to 100 trillion cells, ten-fold the total number of human cells[6], and the majority inhabits the gut. The intestinal microbiota is widely regarded as a virtual organ that actively influences and mediates several physiological functions. These living microorganisms encode for over three millions genes, the so-called "microbiome", outfitting the human genoma by approximately 150-fold[7], endowing human hosts with a wide range of metabolic functions, which they did not develop on their own. A relationship, often termed symbiosis, has developed between the host and the gut microbiota over millions of years.

The distribution of microorganisms within the GI tract depends mainly on the pH gradient and the oxygen availability (Figure 1). Besides density, the microbial composition differs between these sites. Culture studies show – in the stomach and small bowel – considerably fewer bacteria compared with the large bowel, with marked gradients from the duodenum to distal ileum and from the ileum to the colon. The bacteria are typically Gram-positive aerobes proximally and Gram-negative and Gram-positive anaerobes (as well as facultative anaerobes) in the terminal ileum. There are also significant differences between the microbiota present in the gut lumen and the microbiota attached to and embedded in the mucus layer of the GI tract[8].

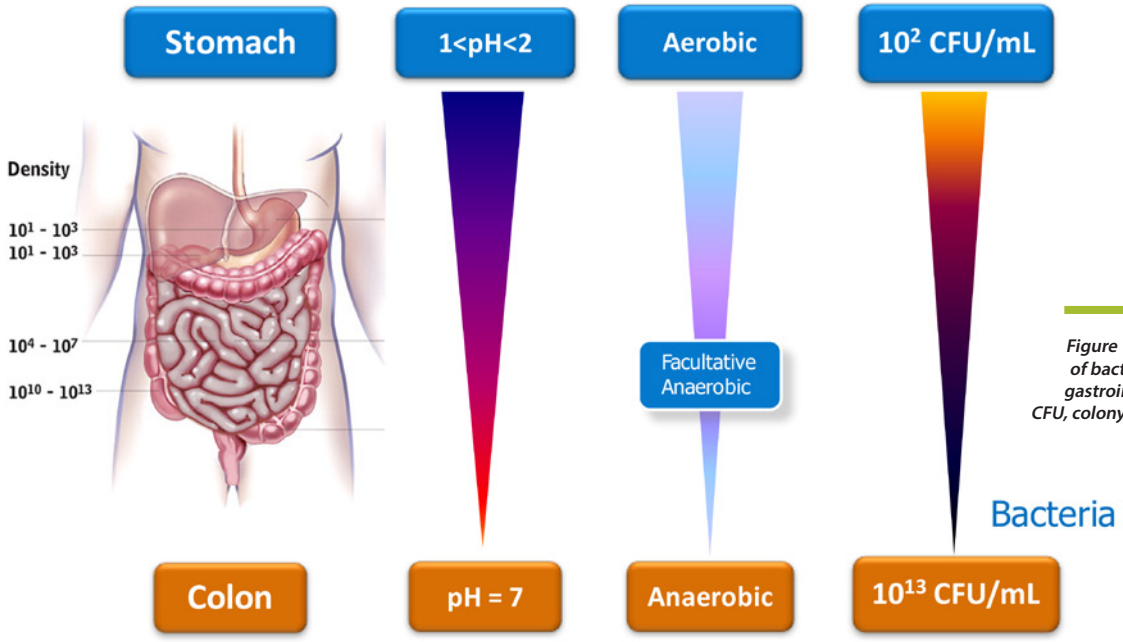

Figure 1: Distribution of bacteria along the gastrointestinal tract. CFU, colony-forming unit.

Esophageal microbiota

The esophagus, unlike the oral cavity, stomach and colon, does not retain food contents. Studies using culturing methods have suggested that the esophagus is either sterile or contains only a few transient microbes originating from the oropharynx by swallowing or from the stomach by gastroesophageal reflux[9].

Culture-based studies mainly used luminal washes of esophageal contents and their results demonstrated that *Streptococcus viridans* may be the most numerous micro-organism in both the healthy esophagus and the oropharynx. Culture-independent methods have recently been used more frequently to characterize the diversity of the microbiota in the esophagus[9]. Pei and colleagues[10] investigated the composition of microbiota in the normal distal esophagus using broad-range 16S rDNA polymerase chain reaction (PCR). They confirmed that the majority of esophageal microbiota were known and cultivable; and found that Streptococcus, Prevotella and Veillonellacee were the most prevalent genera in esophageal biopsies, a finding confirmed also by using a novel device (the Enterotest™ capsule) to sample the microbiome in histologically normal esophageal mucosa[9].

Gastric microbiota

The primary function of the stomach is to prepare food for digestion and absorption by the intestine. Acid production is the unique and central component of the stomach's contribution to the digestive process. The stomach secretes gastric juice, composed mainly of proteolytic enzymes and hydrochloric acid, providing an environment necessary for denaturing of proteins and facilitating the absorption of nutrients. Gastric acid also limits the quantity of microorganisms entering the small intestine and reduces the risk of infection by pathogens. Historically, the prevailing view has been that the stomach is essentially sterile because of its acidic milieu. However, with the discovery of *H. pylori,* it is now known that the stomach can support a bacterial community with hundreds of phylotypes, and while pH values <4 prevent bacterial overgrowth, the acidic milieu is not capable of sterilizing the stomach[11].

Although there is considerable variation in the gastric microbiota between individuals at the genus level, the most prominent phyla detected in the stomach are Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Fusobacteria, the relative abundance of which is influenced by the presence of *H. pylori,* the most dominant species. In the absence of *H. pylori,* analysis consistently notes the presence of *Streptococcus* spp., possibly originating from the oral or nasal cavities and which appear to be the most abundant genus[9, 12, 11].

Intestinal microbiota

Recent studies demonstrate a so far unimagined complexity of the human gut microbiota, with hundreds of phylotypes, of which 80% remain uncultured[8]. Of the 10 bacterial phyla detected in the gut the Firmicutes, Bacteroidetes and Actinobacteria predominate, of which the Firmicutes is the most dominant and diverse phylum in the GI tract. Facultative anaerobes account for <0.1% of the total bacteria detected in fecal samples. Human GI tract microbiota can be divided into three robust clusters called enterotypes, formed by groups of species that jointly contribute to their respective preferred community composition. These enterotypes do not vary by patient characteristics, such as nation, gender, age or body mass index. While most studies used fecal material, this does differ somewhat from the bacteria adherent to the mucosa, which are likely to interact most strongly with the host[8].

Babies start life with sterile intestines, which are rapidly colonized by bacteria from their immediate environment, most importantly their mother's vagina and gut[13]. Early colonizers of the neonatal gut are mainly aerobes (such as staphylococci, streptococci and enterobacteria), while late colonizers are strict anaerobes (such as eubacteria and clostridia) as the total microbiota become more complex, more stable and converge to a common pattern[8, 13]. The microbiota continue to evolve until adulthood with a gradual increase in *Bacteroides* spp., a decline in *Lactobacillus* spp. after the age of five and a decline in *Bifidobacterium* spp. in late teenage. Changes also occur in extreme old age when *Bacteroides* spp. decrease while *Enterococcus* spp. and *Escherichia coli* increase (Figure 2)[8]. Very recently, it was found that both placenta and amniotic fluid harbor a distinct microbiota, characterized by low richness, low diversity and the predominance of Proteobacteria[14]. Based on these data, it was proposed that the stepwise microbial gut colonization process might be initiated prenatally by a distinct microbiota population in the placenta and amniotic fluid. The link between the mother and the offspring is continued after birth by microbes present in breast milk. Further studies are obviously needed to substantiate these findings.

Both intrinsic and extrinsic factors can affect the distribution and composition of the intestinal microbiota (Table 1). A number of host mechanisms participate in gut microbiota modulation, including gastric acid secretion, fluid, systemic and local immunity and antimicrobial peptide production as well as GI motility. Drugs that block acid secretion and affect GI motility can indirectly alter the microbiota. Antibiotics, depending on spectrum and dosage, will directly influence microbiota composition, also affected by dietary modifications, including probiotic and fiber supplements[15]. Finally, NSAIDs are known to increase intestinal bacterial population and change the relative abundance of phyla[16].

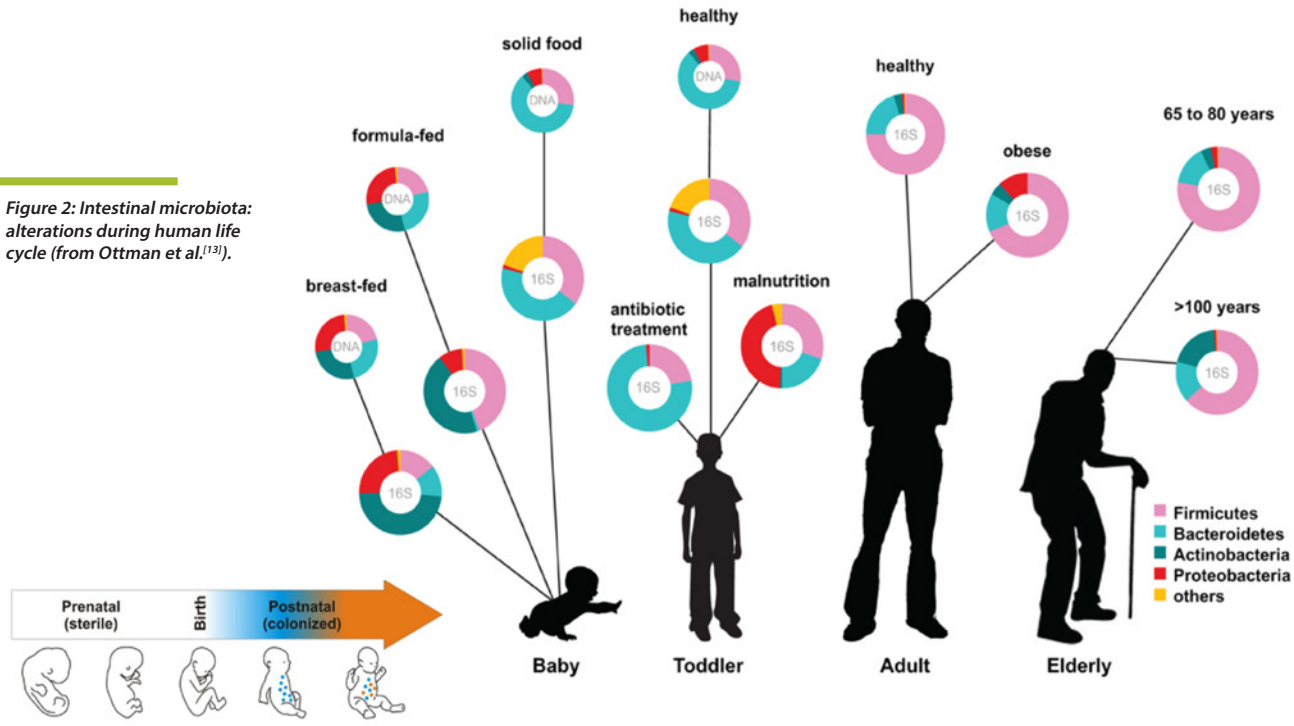

Figure 2: Intestinal microbiota: alterations during human life cycle (from Ottman et al.[13]).

Table 1

Intrinsic and extrinsic factors affecting distribution and composition of gut microbiota

Intrinsic Factors	Extrinsic Factors
Gastric acid	Diet, pre- and probiotics
Oxygen availability	Antisecretory drugs
GI motility	Systemic and topical antibiotics
Mucus secretion and thickness	Prokinetic compounds
Antimicrobial peptides	Laxatives
Local and systemic immunity	NSAIDs

GI, gastrointestinal;
NSAIDs, nonsteroidal anti-inflammatory drugs

PHYSIOLOGICAL FUNCTIONS OF THE GUT MICROBIOTA

Major functions of the gut microbiota include metabolic activities that result in salvage of energy and absorbable nutrients, important trophic effects on intestinal epithelia and on immune structure and function, and protection of the colonized host against foreign microbes[17, 18]. Dysbiosis, changes in microbiota structure, has been linked to inflammatory, functional and metabolic disorders such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and nonalcoholic fatty liver disease (NAFLD) as well as nonalcoholic steatohepatitis (NASH) and even cancer[6, 19-21]. However, it is still not clear whether these changes are a contributing factor or a result of the disease. Nevertheless, bacteria are also useful in promotion of human health. Probiotics and prebiotics are indeed known to have a role in prevention or treatment of some GI diseases[22, 23].

GUT MICROBIOTA IN DISEASE AND THE EFFECT OF ACID SUPPRESSION

Esophageal microbiota

Despite the esophageal microbiota in health is represented by non-pathogenic microorganisms, under certain disease conditions, several pathogenic microorganisms, such as *Candida albicans, Cryptococcus* or *Herpesvirus,* can infect the esophagus[9].

Compared with healthy subjects, the esophageal microbiota is altered in patients with esophagitis or Barrett's esophagus (BE). Indeed, the normal esophagus exhibited a pattern dominated by Streptococcus while biopsy specimens from patients showed a pattern dominated by gram-negative anaerobes or microaerophilic bacteria, which can contribute to esophageal inflammation through activation of Toll-like receptor 4 (TLR4) and consequent overexpression of inflammatory cytokines[9].

Continuous maintenance therapy is indicated in patients with Barrett's esophagus of any length, owing to the potential chemopreventive activity of PPIs against neoplastic transformation, a feature advocated by the American College of Gastroenterology[24] and American Gastroenterological Association[25] but denied by the British Society of Gastroenterology[26]. Indeed, a recent meta-analysis of observational studies showed that PPI use is associated with a 71% reduction in risk of esophageal adenocarcinoma and/or high-grade dysplasia in this patient population[27].

The mucosal associated microbiota of the distal esophagus, which is altered in patients with esophagitis or BE[9], is further modified by PPIs[28]. Acid suppression triggered a significant increase in distal esophageal Lachnospiraceae, Comamonadaceae, and unclassified clostridial families. The family Methylobacteriaceae, which were increased in gastric aspirates among patients with BE/esophagitis before PPIs, were highly depleted in these patients after PPI therapy[28].

Gastric microbiota

The acidity of the stomach distinguishes the gastric niche from the rest of the human GI tract and determines the composition of the gastric flora. Gastric acidity allows *H. pylori* to thrive and is also influenced by the presence of *H. pylori*[11]. The organism is able to survive over a wide pH spectrum. It is found within the gastric mucus layer, deep within the mucus-secreting glands of the antrum, attached to cells, and even within cells[29].

Since Warren and Marshall first described the infectious etiology of peptic ulcer disease in 1984[30, 31], a great deal of evidence has accumulated to suggest that *H. pylori* eradication therapy cures peptic ulcers[32-34] and can be beneficial also to other *H. pylori*-related diseases[35]. Since the organism must be eradicated from each of the above potential niches, doing it is a daunting task for any single antibiotic. Initial attempts to cure the infection showed that the presence of antibiotic susceptibility *in vitro* did not necessarily correlate with successful treatment. It was rapidly recognized that therapy with a single antibiotic led to a poor cure rate and various antimicrobial mixtures were tried resulting in several effective combinations of antibiotics, bismuth, and antisecretory drugs[36].

PPIs display several pharmacological actions that give them a place in any eradication regimen[29], that is:

- they exert an antibacterial action against *H. pylori*;
- by increasing intra-gastric pH, they allow the microorganism to reach the growth phase and become more sensitive to antibiotics such as amoxicillin and clarithromycin;
- they increase antibiotic stability and efficacy;
- and, by reducing gastric emptying and mucus viscosity, they increase the gastric residence time and mucus penetration of antimicrobials.

Acid suppression with PPIs alone decreases *H. pylori* abundance and, in antrum-predominant infection, shifts the location of the microorganism to the corpus; meanwhile, corpus-predominant can cause atrophic gastritis and achlorhydria[29].

Reduction of gastric acid secretion increases the risk of bacterial overgrowth and also influences the composition of intestinal or oral microorganisms, including those organisms causing disease and those with nitrosating ability that are not regularly cultured from a normal, healthy stomach[11]. Individuals infected with *H. pylori* have greater pH changes with PPIs than do uninfected individuals[29] and they are consequently more susceptible to overgrowth. Bacterial enteropathogens *(Salmonella, Campylobacter jejuni, diarrhogenic E. coli, Shigella, Vibrio cholerae, Listeria and C. difficile)* differ as to their acid resistance and pathogenic potential in face of acid suppression. A detailed analysis of the current knowledge in the field is presented in the thoughtful review of Bavishi & DuPont[37].

Hypochlorhydria induced by acid suppression is associated with higher levels of gastric nitrites and an increased risk of gastric cancer[11]. Studies have reported a logarithmic relationship between intragastric pH and median bacterial counts in gastric juice and increased risks for enteric infections, including *C. difficile* infection (CDI), and bacterial-induced diarrhea.

In a recent study[38], a significant correlation was observed between the pH value and the cultured bacterial count in all the samples of gastric fluid (Figure 3), thus indicating that the increase in the bacterial count in PPI-users could be due to low gastric acidity. The comparison between PPI-users and PPI-nonusers found that bacterial cell number in the gastric fluid from PPI-users increased approximately 1000 times using culturing methods, whereas the bacterial number and composition were nearly identical between the two groups using quantitative PCR and a similarity search based on 16S profiling. These results suggest that the gastric microbiota is of salivary origin and are consistent with the idea that the bacterial overgrowth induced by antisecretory drugs may be due to a lack of killing rather than proliferation of the bacteria in the acid-suppressed stomach.

It is worthwhile mentioning that naturally occurring bacteria, some of which are acid-producing and contain ATPase enzymes, have also been found in the stomach, upper GI tract, and oral cavity. Likewise, a number of fungi are known to inhabit the human body and some of these fungi contain H^+-ATPase enzymes. Recent papers suggested that PPIs might affect these microorganisms also directly targeting their proton pumps[39]. However, this effect is not fully understood and more research is needed to elucidate the mechanisms by which PPIs affect microbial population.

In susceptible individuals, chronic *H. pylori* infection can leads to multifocal atrophic gastritis, gastric epithelial dysplasia, and gastric cancer[11]. Gastric cancer is a multifaceted disease with different etiologies, genetic changes and phenotypes. However, *H. pylori* infection is the single most important risk factor for gastric cancer, as it causes chronic inflammatory changes in the gastric mucosa, followed by pre-neoplastic changes such as atrophy and intestinal metaplasia (the so-called Correa's cascade)[11]. Since the risk of gastric cancer increases during these intermediary diseases, it is necessary to interrupt the progression by eradicating the *H. pylori* infection that can halt or even regress some of the mucosal changes. Indeed, a recent meta-analysis demonstrated the benefit of eradication in reducing gastric cancer[40]. As a consequence, a recent global conference held in Kyoto came to a consensus that recommended early eradication of the *H. pylori* infection to enhance the cancer prevention[41].

Other data suggest that *H. pylori* is not the only gastric microorganism that contributes to dysplasia and gastric cancer. In patients with gastric malignant neoplasia, *H. pylori* decreases in abundance and there is a shift toward Streptococci genera that are not often found in normal individuals[42]. It might be therefore possible that long-term PPI use, in *H-pylori*-negative patients or in those eradicated, could promote gastric cancer pathogenesis by causing non-*H pylori* gastric dysbiosis, which can perpetuate the Correa's cascade.

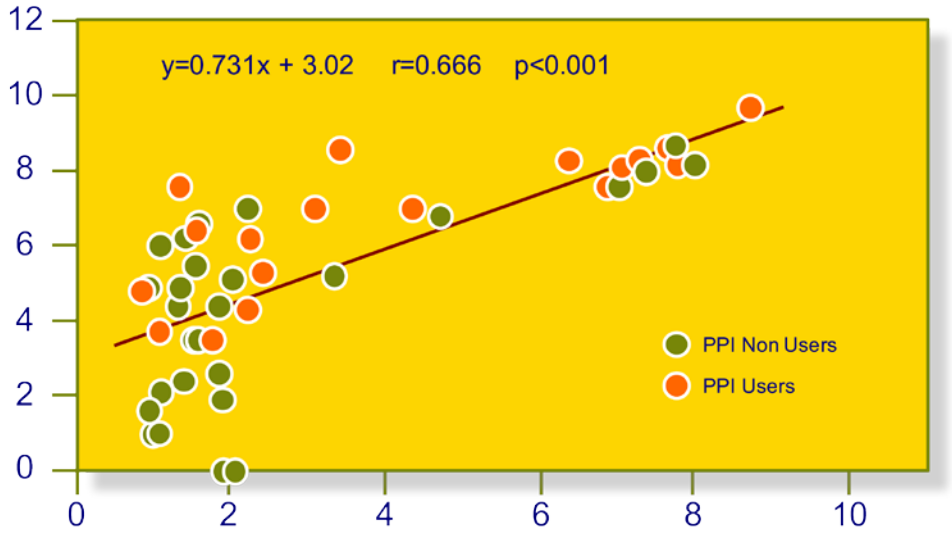

Figure 3: Relationship between the pH value and the bacterial counts in gastric fluid using a culturing method (from Tsuda et al.[38]). PPI, proton pump inhibitor. The pH value (horizontal axis) and the bacterial count (vertical axis) according to a culturing method in the GF of each subject were plotted by a circle to evaluate Spearman's correlation coefficient.

Intestinal microbiota

Changes in microbiota composition have been found in several inflammatory (like IBD), functional (e.g. IBS) and metabolic disorders (such as NASH and NALFD).

Recent advances in DNA sequencing and analysis have revealed many features of dysbiosis in IBD. Reductions in overall gut microbial diversity, decreases in Firmicutes and Bacteroidetes, and increases in Proteobacteria and Actinobacteria were observed. Reduced diversity was also found in inflamed compared with non-inflamed tissue, even within the same patient[18]. A recently published large-scale study of newly diagnosed, treatment-naïve patients with Crohn's disease (CD) showed, in ileal and rectal biopsies, increased Enterobacteriaceae, Pasteurellaceae, Veillonellaceae, and Fusobacteriaceae, and decreased Ersipelotrichales, Bacteriodales, and Clostridales[43]. Importantly, these differences were not readily detected in fecal samples. The decrease in particular microbes may lead to diminished protective effects provided by these gut bacteria that exacerbate inflammation. For example, *Faecalibacterium prausnitzii* (a microorganism with anti-inflammatory activities) is diminished in ileal biopsies of patients with CD.

Various analytic methods have been employed to examine the upper bowel microbiota, with often discrepant changes noted between IBS and healthy control patients[8]. While culture-based methods showed no consistent results, recent molecular approaches found clear differences between healthy subjects and patients with IBS (Table 2)[44]. The improvement of subjective symptoms after gut microbiota manipulation with prebiotics, probiotics, or antibiotics does suggest that the observed differences are clinically relevant. However, it remains to be determined whether IBS symptoms are caused by alterations in brain signaling from the intestine to the microbiota or primary disruption of the microbiota.

Analysis of stool microbiota by pyrosequencing or qPCR showed decrease in Ruminococcaceae family members and Bacteroidetes in NAFLD and NASH subjects compared with healthy controls. A rise in *Clostridium coccoides* was noted in NASH versus biopsy-proven steatosis[18] while a high prevalence of small intestine bacterial overgrowth (SIBO) could be detected by breath testing in both these conditions (up to 50% of NASH patients).

Wireless capsule pH measurement found similar overall small bowel pH between users and non-users of high-dose PPIs[45], suggesting that the profound effect of PPIs on pH is limited to the stomach and proximal duodenum, with little to no effect on the pH of the distal small bowel. Nevertheless, acid suppression does exert a downstream effect on small intestinal bacterial composition. Indeed, recent investigations have shown that the increase in the quantity and diversity of the gastric microbiota in PPI users is paralleled by an increase in the quantity of bacteria in the small bowel.

Table 2

Reported changes of intestinal microbiota in patients with IBS (from Mayer et al.,[44])

Methodologic Approach	Reported Changes
Culture	↓ Bifidobacteri (belonging to the phylum Actinobacteria), Anaerobes ↓ Lactobacilli (belonging to the phylum Firmicutes) ↑ Enterobacteria, Aerobes
PCR-DGGE/qPCR	↓ Anaerobes, Lactobacilli (in D-IBS) ↑ Aerobes
FISH	↓ Bifidobacteria ↑ Firmicutes
Microarray	↓ Bacteroidetes, Bifidobacteria ↑ Firmicutes
16S-pyrosequencing	↓ Bacteroidetes, Bifidobacteria, Actinobacteria ↑ Firmicutes, Proteobacteria

D-IBS, irritable bowel syndrome with diarrhea; FISH, fluorescence in situ hybridization; PCR-DGGE, polymerase chain reaction denaturing gradient gel electrophoresis; qPCR; quantitative polymerase chain reaction.

Several recent studies have evaluated the effect of acid suppression treatment on intestinal microbiota by analyzing fecal samples of PPI-treated patients through the use of *omics* techniques[46-50]. Almost all the studies found a significant reduction in microbial diversity after (even short-term) use of PPIs. In an open-label cross-over trial[47], significant changes

in taxa associated with CDI (increased Enterococcaceae and Streptococcaceae, decreased Clostridiales) and taxa associated with SIBO (increased Micrococcaceae and Staphylococcaceae), were observed during PPI use. Population studies[48, 49] found that multiple oral bacteria were over-represented in the fecal microbiota of PPI-users, including the genus Rothia. In PPI users there is a significant increase in bacterial genera Enterococcus, Streptococcus, Staphylococcus and the potentially pathogenic species *E. coli,* representing changes towards a less healthy gut microbiota (Figure 4). These differences are in line with known changes that predispose to *C. difficile* infection and can potentially explain the increased risk of enteric infections in PPI users. On a population level, the effects of PPIs appear more prominent than the effects of antibiotics or other commonly used drugs.

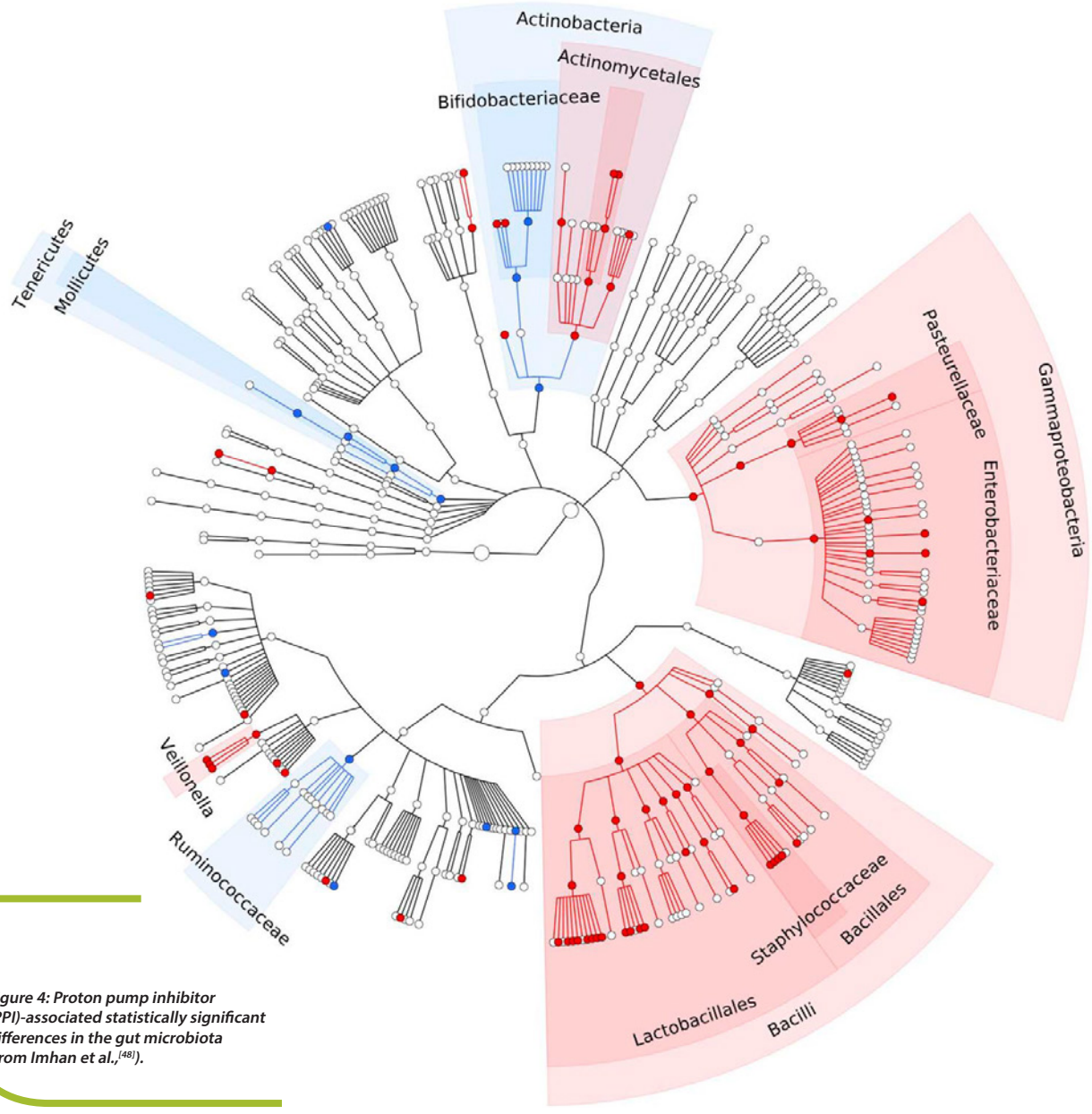

Figure 4: Proton pump inhibitor (PPI)-associated statistically significant differences in the gut microbiota (from Imhan et al.,[48]).

What are the clinical consequences of PPI-induced changes in intestinal microbiota?

As expected from the above taxa modifications, SIBO and CDI are frequent in PPI-users, especially in the long-term.

Despite some controversial findings, several studies and a meta-analysis[51] found that PPI use is statistically associated with an increased risk of SIBO, an effect more evident in studies that used duodenal or jejunal aspirate cultures to make the diagnosis. Both fecal and oropharyngeal type of microbes have been observed to contribute to SIBO. This may be related to the lack of destruction of bacteria swallowed from the oropharynx or to the ascending colonization from the intestine.

A very interesting Italian study[52], showed that patients with non-erosive reflux disease (NERD), who received PPI for typical reflux-related symptoms, while getting relief from heartburn and regurgitation, complained of new onset, SIBO-related, bowel symptoms, whose incidence increased over time (Figure 5). Glucose hydrogen breath test was found positive in 11/42 (26%) subjects and - by a *post hoc* analysis - a significant ($p<0.05$) proportion of patients (8/42) met the Rome III criteria for IBS.

Standard-dose PPIs are indicated for patients taking non-selective NSAIDs at risk for upper GI complications (bleeding and perforation) and for those given selective COX-2 inhibitors (coxibs), who have had a previous GI bleeding episode. In both non-selective NSAID and coxib users PPI therapy reduces upper GI symptoms, in particular dyspepsia[53]. The benefits of such therapy are clearly related to acid suppression and - since NSAID-enteropathy is not a pH-dependent phenomenon - it is not expected to take place beyond the duodenum. Recent experimental and clinical evidence actually suggests that PPIs may aggravate NSAID injury in the small bowel[54-56]. The use of PPIs are now considered to be an independent risk factor associated with NSAID-enteropathy[57]. Since gut microbiota has a pivotal role in NSAID-pathogenesis[58, 54], PPIs may potentiate their cytoxicity in the small bowel via a microbiota-mediated effect.

CDI is the most common cause of healthcare-associated infections worldwide. The infection is developed from an endogenous source or from spores in the environment, most easily acquired during a hospital stay. The use of antimicrobials disrupts the intestinal microflora enabling *C. difficile* to proliferate in the colon and produce toxins. Over the past decade, many studies and several systematic reviews and meta-analyses[59-64] have shown an association between PPI use and CDI amongst outpatients, inpatients and patients in intensive care units. In addition, pre-existing PPI therapy may increase the risk of recurrence or death in male patients with a toxicogenic *C. difficile* infection[65].

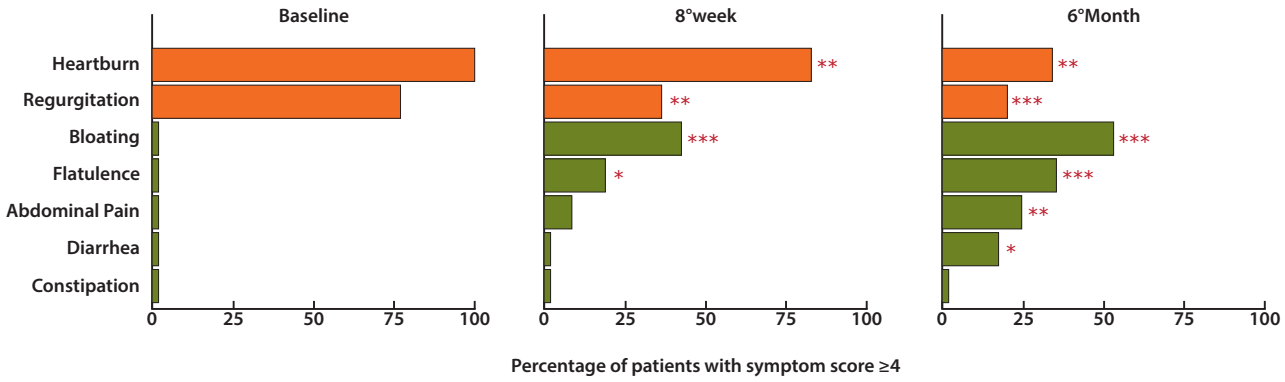

*Figure 5: Symptom pattern changes in patients with non-erosive reflux disease on long-term proton pump inhibitor therapy (from Compare et al.,[51]). *p<0.05 vs baseline; **p<0.01 vs baseline; ***p<0.001 vs baseline; p-values calculated using Fisher's exact test.*

The mechanisms underlying the increased CDI risk in PPI users are not completely understood, but a microbiota-related effect is very likely. *C. difficile* spores are acid resistant[66]; however, when spores and vegetative forms of the microorganism are incubated in fresh gastric juice (pH range 6.4–7.9) from healthy fasting subjects given 7-day esomeprazole (40 mg daily) treatment, heavy growth with an average colony count of more than 10,000 was observed[67, 68]. In addition to this increased viability of ingested vegetative cells in an acid-deficient stomach, PPIs reduce microbial diversity and increase taxa associated with CDI (namely Enterococcaceae and Streptococcaceae, decreased Clostridiales)[48, 49]. Furthermore, PPI users have significantly higher colonic intraepithelial lymphocyte counts compared to non-users[69], suggesting the presence of mucosal inflammation, evidenced also by the increased concentrations of fecal calprotectin[70]. It is well known that IBD patients are at increased susceptibility for CDI compared with the general population[71]. PPIs can also interact with targets other than the gastric H^+/K^+-ATPase. Acting on v-type H^+-ATPase on neutrophils, PPIs inhibit their functions and decrease their bactericidal activity[37]. Several bacteria, including *H. pylori* and *S. pneumoniae,* as well as fungi such as *C. albicans,* contain H^+/K^+-ATPase in their plasma membranes, which are highly homologous to their human counterparts[39], thus influencing microbial growth. Finally, experimental and clinical data suggest that PPIs may increase both gastric[72, 73] and intestinal[74] permeability. All the above interferences with intestinal homeostasis may decrease normal colonization resistance to *C. difficile* and potentially initiate or even exacerbate an ongoing CDI (Figure 6). And indeed, a recent experimental study[75] showed that PPI exposure aggravates *C. difficile*-associated colitis.

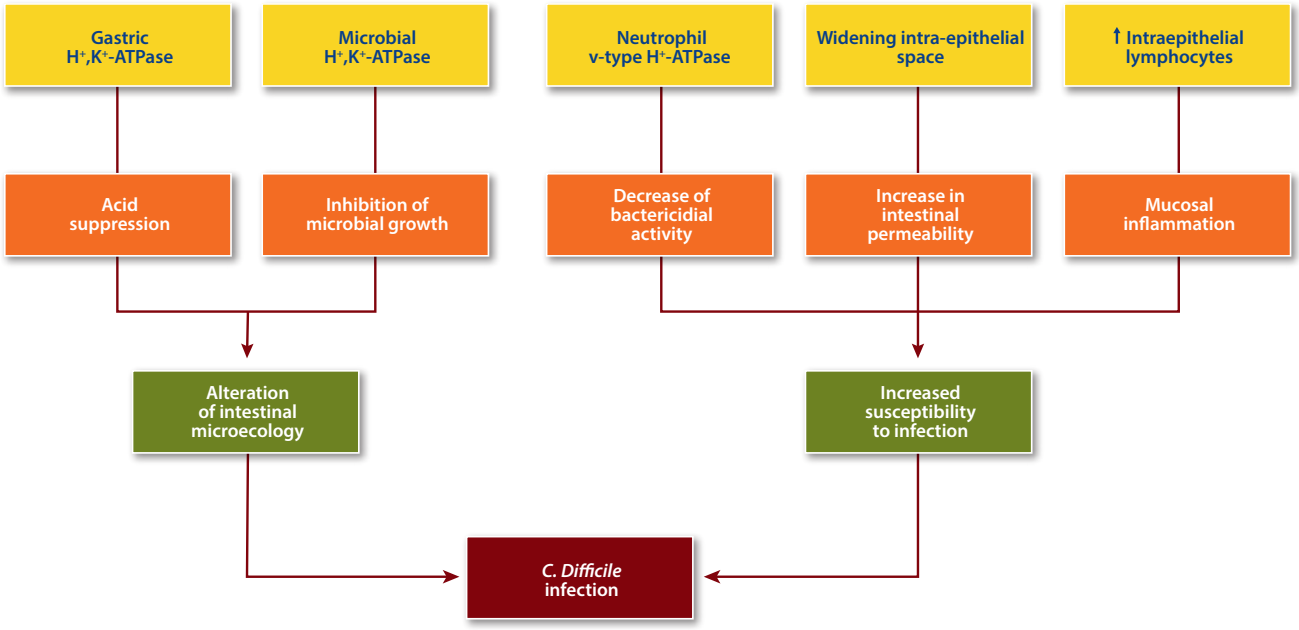

Figure 6: Potential mechanisms underlying the increased risk of C. difficile infection in proton-pump inhibitor users.

CONCLUSIONS AND PERSPECTIVES FOR THE FUTURE

PPIs are among the most widely used prescription drugs. Although concerns have been raised on their long-term safety, current evidence suggests that these concerns are unfounded. Some adverse effects are plausible and predictable. Others are more idiosyncratic, unpredictable, and rare. The described interactions of PPIs with the microbiota of the human gut are not unexpected, but have only recently been well appreciated, thanks to the use of omics tools to study the complex gut ecosystem. Taking into account the large spectrum of influence that gut microbiota has in health and disease, this side effect of acid suppression could have important consequences. An attempt to counterbalance PPI-induced modifications in the diversity and composition of gut microbiota is therefore worthwhile. In this connection, a specifically selected probiotic mixture proved to be capable of significantly reducing bacterial overgrowth in both the stomach and duodenum of PPI short- and long-term users[76].

It is worthwhile emphasizing that - based on the quality of the overall existing evidence - the benefits of PPI treatment outweigh the potential risks in the large majority of patients, especially if PPI use is based on a relevant and appropriate indication[3, 77]. On the contrary, patients treated without appropriate therapeutic indication are *only* exposed to potential risks. Consequently, the overall focus should be on appropriateness of PPI therapy and on a regular assessment of the need for continued PPI treatment.

References

(1) Scarpignato C, Pelosini I, Di Mario F. Acid suppression therapy: where do we go from here? Dig Dis 2006; 24(1–2): 11–46.

(2) Scarpignato C, Pelosini I. Review article: the opportunities and benefits of extended acid suppression. Aliment Pharmacol Ther 2006; 23 (Suppl 2): 23–34.

(3) Savarino V, Di Mario F, Scarpignato C. Proton pump inhibitors in GORD: an overview of their pharmacology, efficacy and safety. Pharmacol Res 2009; 59(3): 135–153.

(4) Sheen E, Triadafilopoulos G. Adverse effects of long-term proton pump inhibitor therapy. Dig Dis Sci 2011; 56(4): 931–950.

(5) Johnson DA, Oldfield EC 4th. Reported side effects and complications of long-term proton pump inhibitor use: dissecting the evidence. Clin Gastroenterol Hepatol 2013; 11(5): 458–464.

(6) Panda S, Guarner F, Manichanh C. Structure and functions of the gut microbiome. Endocr Metab Immune Disord Drug Targets 2014; 14(4): 290–299.

(7) Maccaferri S, Biagi E, Brigidi P. Metagenomics: key to human gut microbiota. Dig Dis 2011; 29(6): 525–530.

(8) Simrén M, Barbara G, Flint HJ, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. Gut 2013; 62(1): 159–176.

(9) Wang ZK, Yang YS. Upper gastrointestinal microbiota and digestive diseases. World J Gastroenterol 2013; 19(10): 1541–1550.

(10) Pei Z, Bini EJ, Yang L, et al. Bacterial biota in the human distal esophagus. Proc Natl Acad Sci U S A 2004; 101(12): 4250–4255.

(11) Hunt RH, Camilleri M, Crowe SE, et al. The stomach in health and disease. Gut 2015; 64(10): 1650–1668.

(12) Walker MM, Talley NJ. Review article: bacteria and pathogenesis of disease in the upper gastrointestinal tract--beyond the era of Helicobacter pylori. Aliment Pharmacol Ther 2014; 39(8): 767–779.

(13) Ottman N, Smidt H, de Vos WM, et al. The function of our microbiota: who is out there and what do they do? Front Cell Infect Microbiol 2012; 2: 104.

(14) Collado MC, Rautava S, Aakko J, et al. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep 2016; 6: 23129.

(15) Albenberg LG, Wu GD. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. Gastroenterology 2014; 146(6): 1564–1572.

(16) Fornai M, Antonioli L, Pellegrini C, et al. Small bowel protection against NSAID-injury in rats: effect of rifaximin, a poorly absorbed, GI targeted, antibiotic. Pharmacol Res 2016; 104: 186–196.

(17) Aziz Q, Doré J, Emmanuel A, et al. Gut microbiota and gastrointestinal health: current concepts and future directions. Neurogastroenterol Motil 2013; 25(1): 4–15.

(18) Chang C, Lin H. Dysbiosis in gastrointestinal disorders. Best Pract Res Clin Gastroenterol 2016; 30(1): 3–15.

(19) Bustos Fernandez LM, Lasa JS, Man F. Intestinal microbiota: its role in digestive diseases. J Clin Gastroenterol 2014; 48(8): 657–666.

(20) Yang AL, Kashyap PC. A clinical primer of the role of gut microbiome in health and disease. Trop Gastroenterol 2015; 36(1): 1–13.

(21) Patel T, Bhattacharya P, Das S. Gut microbiota: an indicator to gastrointestinal tract diseases. J Gastrointest Cancer 2016; 47(3): 232–238.

(22) Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014; 11(8): 506–514.

(23) Lin CS, Chang CJ, Lu CC, et al. Impact of the gut microbiota, prebiotics, and probiotics on human health and disease. Biomed J 2014; 37(5): 259–268.

(24) Wang KK, Sampliner RE, Practice Parameters Committee of the American College of Gastroenterology. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. Am J Gastroenterol 2008; 103(3): 788–797.

(25) Spechler SJ, Sharma P, Souza RF, et al. American Gastroenterological Association technical review on the management of Barrett's esophagus. Gastroenterology 2011; 140(3): e18–52.

(26) Fitzgerald RC, di Pietro M, Ragunath K, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2014; 63(1): 7–42.

(27) Singh S, Garg SK, Singh PP, et al. Acid-suppressive medications and risk of oesophageal adenocarcinoma in patients with Barrett's oesophagus: a systematic review and meta-analysis. Gut 2014; 63(8): 1229–1237.

(28) Amir I, Konikoff FM, Oppenheim M, et al. Gastric microbiota is altered in oesophagitis and Barrett's oesophagus and further modified by proton pump inhibitors. Environ Microbiol 2014; 16(9): 2905–2914.

(29) Scarpignato C and Pelosini I. Antisecretory drugs for eradication of Helicobacter pylori: antibacterial activity and synergism with antimicrobial agents. Progr Basic Clin Pharmacol 1999; 11: 135–178.

(30) Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 1(8390): 1311–1315.

(31) Marshall BJ. The 1995 Albert Lasker Medical Research Award. Helicobacter pylori. The etiologic agent for peptic ulcer. JAMA 1995; 274(13): 1064–1066.

(32) Leodolter A, Kulig M, Brasch H, et al. A meta-analysis comparing eradication, healing and relapse rates in patients with Helicobacter pylori-associated gastric or duodenal ulcer. Aliment Pharmacol Ther 2001; 15(12): 1949–1958.

(33) Gisbert JP, Khorrami S, Carballo F, et al. H. pylori eradication therapy vs. antisecretory non-eradication therapy (with or without long-term maintenance antisecretory therapy) for the prevention of recurrent bleeding from peptic ulcer. Cochrane Database Syst Rev 2003; (4): CD004062.

(34) Ford AC, Delaney BC, Forman D, et al. Eradication therapy in Helicobacter pylori positive peptic ulcer disease: systematic review and economic analysis. Am J Gastroenterol 2004; 99(9); 1833–1855.

(35) Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. Gut 2012; 61(5): 646–664.

(36) Scarpignato C. Towards the ideal regimen for Helicobacter pylori eradication: the search continues. Dig Liver Dis 2004; 36(4): 243–247.

(37) Bavishi C, Dupont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. Aliment Pharmacol Ther 2011; 34(11–12): 1269–1281.

(38) Tsuda A, Suda W, Morita H, et al. Influence of proton-pump inhibitors on the luminal microbiota in the gastrointestinal tract. Clin Transl Gastroenterol 2015; 6: e89.

(39) Vesper BJ, Jawdi A, Altman KW, et al. The effect of proton pump inhibitors on the human microbiota. Curr Drug Metab 2009; 10(1): 84–89.

(40) Ford AC, Forman D, Hunt RH, et al. Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. BMJ 2014; 348: g3174.

(41) Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut 2015; 64(9): 1353–1367.

(42) Cao L, Yu J. Effect of Helicobacter pylori infection on the composition of gastric microbiota in the development of gastric cancer. Gastrointest Tumors 2015; 2(1): 14–25.

(43) Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe 2014; 15(3): 382–392.

(44) Mayer EA, Savidge T, Shulman RJ. Brain-gut microbiome interactions and functional bowel disorders. Gastroenterology 2014; 146(6): 1500–1512.

(45) Michalek W, Semler JR, Kuo B. Impact of acid suppression on upper gastrointestinal pH and motility. Dig Dis Sci 2011; 56(6): 1735–1742.

(46) Seto CT, Jeraldo P, Orenstein R, et al. Prolonged use of a proton pump inhibitor reduces microbial diversity: implications for Clostridium difficile susceptibility. Microbiome 2014; 2: 42.

(47) Freedberg DE, Toussaint NC, Chen SP, et al. Proton pump inhibitors alter specific taxa in the human gastrointestinal microbiome: a crossover trial. Gastroenterology 2015; 49(4): 883–885.

(48) Imhann F, Bonder MJ, Vich Vila A, et al. Proton pump inhibitors affect the gut microbiome. Gut 2016; 65(5): 740–748.

(49) Jackson MA, Goodrich JK, Maxan ME, et al. Proton pump inhibitors alter the composition of the gut microbiota. Gut 2016; 65(5): 749–756.

(50) Clooney AG, Bernstein CN, Leslie WD, et al. A comparison of the gut microbiome between long-term users and non-users of proton pump inhibitors. Aliment Pharmacol Ther 2016; 43(9): 974–984.

(51) Lo WK, Chan WW. Proton pump inhibitor use and the risk of small intestinal bacterial overgrowth: a meta-analysis. Clin Gastroenterol Hepatol 2013; 11(5): 483–490.

(52) Compare D, Pica L, Rocco A, et al. Effects of long-term PPI treatment on producing bowel symptoms and SIBO. Eur J Clin Invest 2011; 41(4): 380–386.

(53) Scarpignato C, Lanas A, Blandizzi C, et al. Safe prescribing of non-steroidal anti-inflammatory drugs in patients with osteoarthritis - an expert consensus addressing benefits as well as gastrointestinal and cardiovascular risks. BMC Med 2015; 13: 55.

(54) Wallace JL. Mechanisms, prevention and clinical implications of nonsteroidal anti-inflammatory drug-enteropathy. World J Gastroenterol 2013; 19(12): 1861–1876.

(55) Fujimori S, Takahashi Y, Tatsuguchi A, et al. Omeprazole increased small intestinal mucosal injury in two of six disease-free cases evaluated by capsule endoscopy. Dig Endosc 2014; 26(15): 676–679.

(56) Washio E, Esaki M, Maehata Y, et al. Proton pump inhibitors increase incidence of nonsteroidal anti-inflammatory drug-induced small bowel injury: a randomized, placebo-controlled trial. Clin Gastroenterol Hepatol 2016; 14(6):809–815.

(57) Marlicz W, Loniewski I, Grimes DS, et al. Nonsteroidal anti-inflammatory drugs, proton pump inhibitors, and gastrointestinal injury: contrasting interactions in the stomach and small intestine. Mayo Clin Proc 2014; 89(12): 1699–1709.

(58) Scarpignato C. NSAID-induced intestinal damage: are luminal bacteria the therapeutic target? Gut 2008; 57(2): 145–148.

(59) Leonard J, Marshall JK, Moayyedi P. Systematic review of the risk of enteric infection in patients taking acid suppression. Am J Gastroenterol 2007; 102(9): 2047–2056.

(60) Janarthanan S, Ditah I, Adler DG, et al. Clostridium difficile-associated diarrhea and proton pump inhibitor therapy: a meta-analysis. Am J Gastroenterol 2012; 107(7): 1001–1010.

(61) Kwok CS, Arthur AK, Anibueze CI, et al. Risk of Clostridium difficile infection with acid suppressing drugs and antibiotics: meta-analysis. Am J Gastroenterol 2012; 107(7): 1011–1019.

(62) Abou Chakra CN, Pepin J, Sirard S, Valiquette L. Risk factors for recurrence, complications and mortality in Clostridium difficile infection: a systematic review. PLoS One 2014; 9(6): e98400.

(63) Deshpande A, Pasupuleti V, Thota P, et al. Risk factors for recurrent Clostridium difficile infection: a systematic review and meta-analysis. Infect Control Hosp Epidemiol 2015; 36(4): 452–460.

(64) Furuya-Kanamori L, Stone JC, Clark J, et al. Comorbidities, exposure to medications, and the risk of community-acquired Clostridium difficile infection: a systematic review and meta-analysis. Infect Control Hosp Epidemiol 2015; 36(2): 132–141.

(65) Dos Santos-Schaller O, Boisset S, Seigneurin A, et al. Recurrence and death after Clostridium difficile infection: gender-dependant influence of proton pump inhibitor therapy. Springerplus 2016; 5: 430.

(66) Rao A, Jump RL, Pultz NJ, et al. In vitro killing of nosocomial pathogens by acid and acidified nitrite. Antimicrob Agents Chemother 2006; 50(11): 3901–3904.

(67) Jump RL, Pultz MJ, Donskey CJ. Vegetative Clostridium difficile survives in room air on moist surfaces and in gastric contents with reduced acidity: a potential mechanism to explain the association between proton pump inhibitors and C. difficile-associated diarrhea? Antimicrob Agents Chemother 2007; 51(8): 2883–2887.

(68) Sharara A, ElHajj II, Maasri K, Hashash JG, Araj GF. W1276 the effect of acid, pepsin and gastric juice on the in vitro growth and sporulation of Clostridium difficile. Gastroenterology 2008; 134(4 Suppl 1): A-670.

(69) Yu YH, Han DS, Choi EY, et al. Is use of PPIs related to increased intraepithelial lymphocytes in the colon? Dig Dis Sci 2012; 57(10): 2669–2274.

(70) Poullis A, Foster R, Mendall MA, et al. Proton pump inhibitors are associated with elevation of faecal calprotectin and may affect specificity. Eur J Gastroenterol Hepatol 2003; 15(5): 573–574.

(71) Reddy SS and Brandt LJ. Clostridium difficile infection and inflammatory bowel disease. J Clin Gastroenterol 201; 47(8): 666–671.

(72) desMullin JM, Valenzano MC, Whitby M, et al. Esomeprazole induces upper gastrointestinal tract transmucosal permeability increase. Aliment Pharmacol Ther 2008; 28(11–12): 1317–1325.

(73) Murray LJ, Gabello M, Rudolph DS, et al. Transmucosal gastric leak induced by proton pump inhibitors. Dig Dis Sci 2009; 54(7): 1408–1417.

(74) Singh DP, Borse SP, Nivsarkar M. A novel model for NSAID induced gastroenteropathy in rats. J Pharmacol Toxicol Methods 2016; 78: 66–75.

(75) Hung YP, Ko WC, Chou PH, et al. Proton-Pump inhibitor exposure aggravates Clostridium difficile-associated colitis: evidence from a mouse model. J Infect Dis 2015; 212(4): 654–663.

(76) Del Piano M, Anderloni A, Balzarini M, et al. The innovative potential of Lactobacillus rhamnosus LR06, Lactobacillus pentosus LPS01, Lactobacillus plantarum LP01, and Lactobacillus delbrueckii Subsp. delbrueckii LDD01 to restore the «gastric barrier effect» in patients chronically treated with PPI: a pilot study. J Clin Gastroenterol 2012; 46 Suppl: S18–26.

(77) Vakil N. Prescribing proton pump inhibitors: is it time to pause and rethink? Drugs. 2012; 72(4): 437–445.

ROLE OF MUCOSAL MICROBIOME IN THE DEVELOPMENT AND FUNCTION OF MUCOSAL IMMUNE SYSTEM

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ABSTRACT

Mucosal surfaces and the skin are the primary sites of initial biologic interactions between the mammalian host and the external environment. These sites bear the major burden of continuous exposure to diverse spectrum of components in the environment. These include numerous subcellular, unicellular and multicellular organisms, dietary agents and food products, and numerous other soluble or cellular, air or water borne, natural or generated products. The development of the different functional aspects of innate and adaptive immunity in the mucosal surfaces and the skin are the principal mechanisms of mammalian defense to maintain effective homeostatic balance between the host and the external environment. The innate immune functions are mediated by a number of host specific microbial or pathogen specific recognition receptors, designed to recognize unique microorganism associated molecular patters, essential to the molecular structure and often to the survival of the microorganisms. The major components of specific adaptive immunity in the mucosal surfaces include the organized antigen-reactive lymphoid tissue follicles in different inductive mucosal sites and, the effector sites of the lamina propria and sub epithelial regions which contain lymphoid and plasma cells derived by the homing of antigen sensitized cells from the inductive sites. The acquisition of environmental microbiome by the neonate in its mucosal surfaces and the skin begins before or immediately after birth has been shown to play a critical and complex role in the development of mucosal immunity and lifelong immunologic homeostasis of the host. This report provides a brief overview of the mammalian microbiome in different vertebrate species, and highlights the role of mammalian microbiome in the evolution and functional development under normal physiologic conditions and during certain pathologic or disease states.

Key words: childhood vaccines; gut microbiome; innate immunity; mucosal immunity; vertebrate microbiome.

INTRODUCTION

The evolution of life on our planet began with the appearance of replicating subcellular and non-nucleated unicellular (Prokaryotic) organisms about 4 billion years ago, followed by the development of nucleated (Eukaryotic) unicellular organisms about 2 billion years ago. Over the past 1 billion years, these milestones resulted in relatively rapid evolution of numerous multicellular organisms, including man. Recent studies have suggested that the evolution of guanylate kinase-protein intervention domain (GK-PID), a molecule designed to link proteins during cell division may be the most critical component of the framework underlying the organization and eventual evolution of multicellular life forms[1].

Virtually all multicellular organisms live in a complex relationship with environmental microbiome, a diverse spectrum of unicellular organisms and other subcellular life forms. These include: viruses, bacteria, archaea, protista and possibly other still to be defined replicating life forms. Most mammalian species are heavily colonized by different components of the microbiome. The mammalian host-microbiome relationships begins shortly after (and possibly before) birth, and is intimately linked to the development of long term and complex mutual biologic interactions, critical to their mechanisms of homeostasis and survival. While the functional nature of these interactions is mostly complimentary and mutually beneficial, their effects under certain circumstances are competitive and potentially harmful to either the host or the microbiome.

Mammalian mucosal surfaces and the skin represent the major sites for the resident colonization by the environmental microbiome. Its interactions with mammalian cellular mass are largely responsible for the effective functional development of immunologic and other homeostatic mechanisms in the host, as well as the functional integrity of the resident microbiome.

This report will provide a very brief overview of the environmental microbiome and its impact on the development and function of innate and adaptive immune responses in the mammalian mucosal surfaces.

MAMMALIAN MICROBIOME

The overall content and the spectrum of the mucosal microbiome is currently based on the detection of microorganism-specific genomic sequences in the host. Based on their taxonomic profiles investigated to date, mammalian microbiome is largely composed of organisms of the following phyla: Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and Synergisteles. The relative contribution of different phyla varies considerably and is influenced by numerous environmental factors.

Skin microbiome

The entire surface of the mammalian skin constitutes the largest and the single most visible organ of human and other mammalian species. It is estimated that over one billion bacteria reside in a square cm of skin surface and its associated appendage and glandular tissues[2, 3].

The skin microbiome normally exhibits marked diversity and quantitative variability. It appears to shift constantly in response to external environmental factors and the host internal milieu. Majority of phyla of established or resident microbiome belong to Actinobacteria, Bacteroidetes, Firmicutes or Proteobacteria based on 16S ribosomal RNA gene sequencing. The distribution of different organisms is site dependent, between, moist areas and creases (staphylococcus and Corynebacterium species), sebaceous glands (propionobacterium species of Actinobacteria phyla) and in dry regions (most diverse and varying representation of all 4 phyla) of the human body[4-6]. The long-term microbial residents are a relatively fixed group of organisms found routinely on normal skin. The transient organisms do not generally reside constantly in predetermined skin sites. However, under certain pathologic states, many organisms exhibit altered colonization patterns, proliferate locally and are associated with disease. The skin is initially colonized at the time of birth with a microbiome of very low diversity and is largely shaped by the method of delivery. Subsequently, the microbiome acquires more specificity and diversity at different body sites harboring over 150 species of different phylotypes[6]. However, the precise nature of colonization by different viruses, and other subcellular and unicellular organisms in the skin still remains to be fully elucidated.

Mucosal microbiome

The mucosal surfaces of the human neonate are essentially sterile at birth, but begin to be colonized by microorganisms shortly thereafter. The initial acquisition of microflora occurs via the maternal genital tract following routine vaginal delivery, followed by organisms in the maternal gastrointestinal tract and other maternal mucosal surfaces, maternal skin, and finally by the process of breast feeding. Subsequently, the neonatal microflora is supplemented with other environmental microorganisms, from other humans, pets and other animal species and, from organisms in the soil. Microbial colonization by physiologic resident (normal) flora is usually complete within 1–2 weeks of postnatal life. The final composition of mucosal microbiome is a function of breast-feeding, nature of exposure to external environment and the cultural patterns of living in early childhood in different parts of the world. Breast fed infants develop intestinal microbiome which is rich in Bifidobacterium species. Subsequently, the microflora becomes more diverse with a predominance of Firmicutes and Bacteroides species. In addition to breast-feeding, the mucosal microflora is also significantly affected by diet and other aspects of mucosal microenvironment including, antibiotics, other environmental macromolecules and supplemental formula feeding. Although the qualitative and quantitative aspects of microbial diversity exhibit marked fluctuations under different environmental conditions during the first year of life, the mucosal microflora, once established remains surprisingly stable, and unique and specific to each individual. As pointed out earlier, the primary source of neonatal colonization is the mother. The maternal genital tract contains over 10^{12} organisms representing many aerobic and anaerobic species with predominance of Coliform, Streptococcal species, Gram positive anaerobes, Lactobacilli, Prevotella and Sneathia species. Human intestinal lumen contains of over 10^{14} bacteria representing as many as 2000 species, with over 160 species per individual[7]. It is estimated that human gut microbiota contains more genes than the entire human genome. Recent observations have suggested that human breast milk contains over 700 microbial species which exhibit significant changes in quality and quantity after establishment of lactation. The microbiome in the early colostrum appears to be most diverse, with high numbers of Streptococcus, Lactococcus, Leuconostoc, and Weisella and Staphylococcus species. Subsequently, milk obtained after establishment of lactation exhibits a microbiome which is somewhat limited to the organisms in Prevotella, Veillonella and Leptotrichia species.

The microbial content of milk exhibits significant differences in mothers who undergo C-section for the delivery and in those mothers who exhibit malnutrition. The surface area of the human mucosal surfaces and the skin is extremely large. However, the relative load of the microorganisms and the qualitative nature of microbiome is strikingly different in different human mucosal surfaces. The microbial load appears to be lowest in the lungs, stomach and small intestine[8]. It is estimated that once fully colonized, there are over 200 trillion or more microorganisms

representing over 2000 bacterial species residing in and on each human being[9]. Of these, only about 150 species have been cultured and studied *in vitro* to date.

In addition to the environmental conditions, which affect the diversity and composition of mucosal microbiome, there is strong evidence for significant alterations in mucosal microbiome in ageing subjects. The microbiome appears to shift towards a Bacteroides predominant pattern in the elderly, with significant loss of diversity in the core microbiota in frail older subjects[10].

The mucosal surface of the gastro intestinal tract is a large and constantly exposed to heavy loads of environmental microbial agents, dietary macromolecules and other environmental antigens. While human gut microbiome is replete with Clostridia species of Firmicutes phyla, with a small contribution from species of Bacteroides and Proteobacteria, studies carried out in other vertebrate animals such as fish, frogs and mice have demonstrated strikingly different patterns of gut microbiome (Table 1)[11]. The implications of different patterns of gut microbiome in different species have also been related to the striking differences in the nature of immune responses in different vertebrate species studies to date[12].

Table 1

Distribution and taxonomic profile of gut microbiome in different vertebrate species[11, 12].

	Relative proportion of organisms (%) in the gut			
	Fish	Frog	Mice	Human
Proteobacteria	60	7	2	1
Fusobacteria	35	<1	-	-
Firmicutes (clostridia)	5	78.5	60.0	88
Bacteroidetes	<1	12.5	37.5	10
Actinobacteria	<1	-	-	1
Synergistietes	<1	1.0	-	-
Other	<1	1	<1	<1

EVOLUTION OF MUCOSAL DEFENSES AND IMMUNE RESPONSES

Innate immunity

Mucosal mechanisms of defense include the epithelial barrier, a single layer of epithelial cells in the mucosal surface of the gastrointestinal, respiratory and genitourinary tracts and the skin. These cells represent the primary interface between the external environment and the host. These include terminally differentiated apical cells, cells with microvilli (M cells), ciliated cells, intraepithelial lymphocytes (IEL), intestinal mononuclear phagocytes (IMP), including dendritic cells (DC), macrophages, and neutrophils. They are differentially equipped for specific functions such as, antigen uptake, antigen processing and delivery, mucin production, elimination of microbial, dietary or other environmental macromolecules; receptor mediated microbial transport, and as epithelial barrier functions, against a variety of environmental agents.

Mucosal epithelium represents an essential component of the host immunity. The M cells in the gut and follicle-associated epithelium (FAE) are derived from stem cells in crypt regions following cellular differentiation into crypt and follicular epithelium.

In addition to the epithelial cell barrier, the major mechanisms of innate mucosal immunity include, microbial and other pathogen recognition receptors (PRR). These receptors are designed to recognize unique pathogen and other microbial- associated molecular patterns (PAMP), which are often essential for the survival of the organisms. The PRR include, mannan-binding lectin, LPS binding protein, C-reactive protein, macrophage-mannose receptor, macrophage scavenger receptors, macrophage receptor with collagenous structure (MARCO), multiple Toll-like receptors (TLR 1 to TLR 13), ds RNA-activated protein, CD_{14}, nucleotide-binding oligomerization domain (NOD), RP 105 (DC180, Ly78, and MDI) and MD2 (Ly-96). Specific microbial ligands for these intra-cytoplasmic and trans-membrane receptors have been well characterized and their interaction with specific microbial ligands elicit diverse biologic functions, including NF-Kβ activations, apoptosis, opsonization, complement activation and specific signaling for B and T cell responses[12].

The biologic functions of different components of innate immunity especially the PRR are genetically pre-determined and antigen independent. The relative number of receptors in the PRR family is limited to few hundred to thousand in number. However, they play a critical role in, regulating the microbial content in the mucosa, epithelial proliferation, mucosal permeability and interaction with specific adaptive immune responses[11, 12].

Adaptive immunity

Antigen specific antibody (B cell) and cell mediated (T cell) immune responses in the mucosal surfaces begin to appear as early as 15 weeks of gestational life.

Briefly, B cell subsets (Bla: CD5+ CD19+ CD45+, Cb11b+ SIgM (high) SIgG (low): B_1b: CD5- CD116+ and B_2 (CD9+ CD45R+) appear in different mucosal sites between 14–17 weeks of gestation.

However, over 90% of cord blood B cells are of B1 phenotype, but represent only about 25–30% of adult B cells. B_1 B cells interact with innate immune cells and maintain critical interaction with the mucosal microbiome. These cells eventually differentiate into polyclonal IgM secretory cells with immediate and broad-spectrum antimicrobial activity. These cells also differentiate into IgA producing cells after migration to the mucosal surfaces including the gut, especially after immunization with viral vaccines[13, 14]. While it has been suggested that B_1 cells may induce memory responses, their role in the evolution and functional aspects of mucosal immunity remain to be determined.

Mucosal associated lymphoid tissue (MALT) and common mucosal immune system

The lymphoid tissue associated with mucosal surfaces of gut, respiratory tract, genital tract and the mammary glands is the largest site of antibody production in the body. As discussed earlier, the mucosal epithelium contains M cells, IEL, DC, monocytes, macrophages, neutrophils, B&T cells and plasma cells. The subepithelial regions contain organized lymphoid follicles containing antigen reactive B and T cells, DC and macrophages, and represent primary induction sites for processing and presentation of antigens, induction of antigen specific lymphoblasts, expression of activated B and T cells and eventually induction of mature antigen-specific plasma cells. The major inductive sites of mucosal immune response include the sub epithelial organized follicles of lymphoid tissues in the gut (GALT), bronchoepithelium (BALT), nasopharynx (NALT), sublingual tissue (SLT), skin (SALT), and possibly the larynx associated lymphoid tissue (LALT)[13].

The cascade of events resulting in the development of antigen specific mucosal immune response begins with antigen sampling by M cells in the mucosal epithelium, followed by activation of IEL, mucosal DC, and regulatory T cells (Tregs), and expression of several cytokines, including IL-5, IL-6, IL-10, IL-23, IL-27, retinoic acid and TGF-β. Induction of Tregs and activation of other T cell subsets, followed by expression of IL-5, IL-6, and IL-10 and expression of IgA plasma cells and production of specific antibody response in the effector sites in the lamina propria of the site of immunization as well as in distant effector sites such as genital tract, and mammary glands. Although exposure to antigens at primary inductive sites elicits a widespread response in other distant mucosal sites, the response is somewhat compartmentalized and site specific, as exemplified by the development of specific responses in the intestine, genital tract and the mammary glands after oral administration of an antigen. On the other hand, intranasal immunization has been shown to induce a response in the lungs and upper airway and in the genital tract with relatively low response in the gut. Local intra-rectal, intra-vaginal or intra-colonic exposure to antigens results in antibody response largely restricted to the site of primary exposure. The restricted nature of the mucosal response appears to be related to expression of unique homing ligands in activated B cells and in different inductive sites. These include α4β7 integrin and CCL25, CCR9, MAdCam1 ligands in the gut[13].

MUCOSAL MICROBIOME AND ITS IMPACT ON MUCOSAL IMMUNE RESPONSE

The mammalian microbiota exhibits diverse and striking effects on the development and function of different aspects of mucosal defenses. Specific microbial components and/or metabolites derived from the mucosal microbiota, including bacteria, viruses and helminthes act on superficial epithelium, IEC, DC, IEL, macrophage and leukocytes to regulate mucosal barrier function and development of effective immune responses. Mucosal microbiome and microbiota-derived factors are essential for activation of pathways underlying the development of IgA antibody production and regulation of the critical balance between effector and regulatory T cells. More recent observations have reinforced the importance of mucosal microbiome in the maintenance of health, prevention of disease in the gut and other mucosal sites and beyond, and in modulating the expression of several systemic disease states[15].

Gut microbiota has been shown to have a significant impact on both innate and adaptive T cell function and development. Mucosal microbiome especially of the gut mucosa has several specific effects on T cells. These include, modulation of CD4+ and other T cell subset balance, differentiation of CD4+ T cells toward Treg cells, modulation of the activation status of γδ T cells in the mucosal lymphoid tissue via their ability to respond to TLR signaling and Th17 production, induction of Foxp3 Treg cells[15].

Gut microbiota has a profound impact on B cell function and mucosal IgA response. Germ free mice conspicuously exhibit marked deficiency of IgA antibody production. Mucosal commensals induce IgA plasma cells to acquire myeloid cell-like phenotypes and develop multifunctional properties with expression of TNFα inducible nitric oxide synthase (IN05), and protection against induced infection with *Citrobacter rodentium* under experimental conditions. Recent observations have also shown that T cell independent switch of IgA B cells is also mediated by gut microbiota via their effect on IEC and IMP to secrete B cell activation factors of TNF family.

Gut commensals have also been found to induce IgA B cell switch through T cell-dependent pathways via induction of Treg cells[15].

As previously discussed, the maturation of mucosal immune system is to a large extent determined by the exposure to and the nature of environmental microflora, beginning shortly before birth and during the early neonatal life. This period is characterized by reduced activity of innate immunity, low complement component levels, impaired IFN-Y, IL-10 production, reduced APC function and Th_1 mediated T cell and enhanced Th_2 mediated T cell subsets, reduced intracellular killing of cell-associated organisms, and delayed expression of cutaneous hypersensitivity reactions[14]. The initial colonization of the normal vaginally delivered neonate occurs from the maternal genital tract, gastrointestinal tract and breast-feeding. The colonization of the neonate during this period is critical to the subsequent long-term character of mucosal immune functions throughout its lifetime.

Epidemiologic studies carried out several years ago[16], demonstrated that breast-feeding was associated with significantly reduced severity of clinical disease and enhanced multiplication of bifidobacteria following naturally acquired rotavirus infection in infants. More recent studies[17, 18], have demonstrated that oral feeding of bifidobacteria was associated with significant reduction in endotoxin levels in the gut, suggesting a possible altered composition of gut microflora after the introduction of such commensals. In other studies, these investigations demonstrated a significant increase in fecal and serum IgA rotavirus specific antibody levels following supplemental feeding with bifidobacteria during acute rotavirus infection[16-18]. Similarly, infection with non-pathogenic strains of salmonella have been found to inhibit activation of genes coding for inflammatory cytokine expression via inhibition of NF-κB activation. Other studies have demonstrated induction of decay-accelerating factor (DAF), which inhibits cytotoxic damage from microbial activation of C-reactive protein, ductin, induction of tolerance to IgE production, expression of genes regulating angiogenesis and other host genes involved in the maturation, nutrient uptake and other metabolic process of xenobiotics[8, 19].

Mucosal microflora also exhibit significant role in the maturation of innate immunity, including the expression of Toll-like receptors 4, and 5, enhanced development of proliferation of different components of organized mucosal lymphoid tissue, induction of and plasticity of IgA response in the gut, shift of Th response towards Th1 type cells, and down regulation of NK T cells and their inflammatory effector functions[8].

Virtually all microbial organisms resident in the human host are acquired from other life forms and a normal human being possesses >100–200 trillion microbial organisms in different mucosal and cutaneous tissue. Benign colonization of the human mucosal surfaces by the microorganism is the rule and long established microflora (commensals) in man is almost always symbiotic, and development of disease is an exception rather than the rule. Acquisition of new microbial colonization may result in local inflammation which often helps terminate infection promptly. However, under certain host or microorganism derived conditions, the interaction can result in chronic inflammation and development of disease and possibly death of the host. There is now increasing evidence to suggest that a strong correlation exists between inflammatory bowel disease (such as ulcerative colitis, Crohn's disease) and altered gut microbiota. Although, transmission of microbiome from the mother to her neonate may have a major role in conferring a distinct pattern of microbial colonization in all subjects, it remains to be determined if the changes in the microbiome observed in IBD, represent cause or the effect of IBD[20].

The influence of mucosal microbiome appears to extend beyond the gut, to other systemic disease states as well. Specific changes in quantitative and qualitative nature of mucosal microbiome have been observed in several distinct clinical disorders. These include allergic diseases, diabetes mellitus, obesity, antibiotic resistance and associated infections with such organisms in systemic or mucosal sites[20, 21]. The microbial alterations observed in these disorders are listed in Table 2.

The observations summarized above provide strong support for the role of mucosal microbiome in the development and function of innate and adaptive mechanism of mucosal immunity, and their possible role in the evolution of several disease states in the gut and beyond.

Table 2

Changes in mucosal microbiome in different clinical disease states[20, 21].

Disease states	Changes in mucosal microbiome and cytokine content	
	Increased	Decreased
Allergic Disorders	Proteobacteria IL-4	Bifidobacteria Clostridia Lactoacilli *H. pylori*
Diabetes mellitus	*Bifidobacteria: Firmicutes ratio* Bacteroides ovatus	Microbial diversity *Clostridia sp.* *Firmicutes*
Obesity	*Firmicutes* *Actinobacteria* TNF-α	*Bacteroides* Inflammatory response
Microbial antibiotic resistance	Resistance gene reservoir in gut microbiome	-
Crohn's Disease Persistent Clostridial infection Depression Autism Specific Disorder	Variable – not fully characterized	

Impact of microbiome on immunization

The implications of mucosal microbiome and immunity must also be applicable to the development of vaccine-induced immunity, especially after mucosal immunization. Recent studies suggest that the diversity and the composition of gut microbiota can influence the efficacy of oral vaccines[23, 24]. Orally administered vaccines may not be highly effective in many parts of the world, This has been observed with immunization with oral polio vaccines especially in the tropical countries with significant malnutrition and heavy microbial overload with non-commensal microorganisms. Limited numbers of studies carried out to date have observed increase, decreased, or no significant changes in the immune responses to a variety of childhood immunization schedules after induced alteration in the mucosal microbiome by ingestion of lactobacillus species or other commensal organisms. Unfortunately limited or no information is available regarding the status of mucosal immune responses in specific qualitative or quantitative alterations in the mucosal microbiome. Available, although limited information has been recently reviewed by Valdez and colleagues[25]. Possible factors involved in the induction of inferior vaccine induced immune responses in such situations are listed in Table 3.

Table 3

Possible factors involved in the development of poor or inadequate immune response to oral and other mucosally administered childhood vaccines[25].

1 • Method of delivery (C-section)
2 • Feeding practice (formula feeding)
3 • Undernutrition - malnutrition
4 • High carbohydrate diet
5 • Early use of antibiotics
6 • Impaired barrier and immune functions
7 • Enteropathy
8 • Chronic mucosal inflammation
9 • Altered mucosal microbiome
10 • Over exposure to pathogens, dysbios – dysfunctional mucosal microbiota

CONCLUSIONS

Based on the information briefly reviewed above, it is clear that human microbiome contributes in a remarkable manner to the early development and functional maturation of the immune system at both systemic and mucosal levels. The mucosal immune system is in a continuous, and as a rule, mutually beneficial or symbiotic relationship with the microbiome. The immunologic mechanisms of the host must also maintain a constant vigil for the presence of pathogenic organisms. Thus, the mucosal microbiome may be involved both in the health of the host, as well as in the induction of disease under certain circumstances

in the mucosal sites as well as systemic sites. The complex nature of host-microbial interactions is regulated at several levels by the microbiota and by the induced host immune responses. The initial events are characterized by the mucosal epithelial cells which sample the microbial organisms, maintain barrier function and contribute to the regulatory function of the mucosal phagocytes (DC and macrophage). The cellular products and specific cytokines released by IEC and iMP appear to directly impact on the functional activity of innate lymphoid cells (ILC). The mucosal microflora and or its metabolic products, can serve as ligands, such as aryl hydrocarbon receptor (Ahr) and influence ILC. In addition to the significant role of microbiota in the induction of innate immune responses, and the cross regulation of microbial-innate lymphoid cell interaction, mucosal microbiota stimulates multiple pathways of B cell activation, which eventually lead to mucosal IgA production[25]. The potential impact of select components of mammalian microbiome is briefly summarized in Table 4. In addition to their impact on ILC, B cell and IEC, recent observations have also demonstrated that mucosal microbiota shapes the differential and functional maturation of mucosal T cells and their subsets.

Finally, recent investigations have suggested that the specific composition and or the nature of diversity of the mucosal microbiome may influence the efficacy of orally administered vaccine against certain infectious agents. However, the mechanism underlying such interactions remain to be defined. Hopefully future studies well provide more specific answers to the role of microbiota in the induction of several disease problems described in this review.

Table 4

Effects of mucosal colonization with select microbial species on mucosal immune function[25].

Segmented filamentous bacteria (SFB)	*B. fragilis*	Clostridium cluster, IV and XIV$_a$	*Sphingomonas yanoikuyae*
Expansion of Th-17 cell in Ileum via production of serum amyloid A (SAA)	Induction of Foxp^3 Tregs in colon via polysaccharide A (PSA), mediated through TLR2	↑ Treg expansion in gut	↑ *Modulation of the phenotype and response of iNKT cells, independent of TLR or IL-12 activation*
Activation of Treg via DC	↑ IL-10 by Treg	↑ TGFβ, MMP2, MMP9, MMP13, IDO, via activation of IEC in colon	
	↓ Th-17 cell expansion in gut	↓ Serum IgE and serum IL-4	
		↑ IL-10	

References

(1) Anderson DP, Whitney DS, Hanson-Smith V, et al. Evolution of an ancient protein function involved in organized multicellularity in animals. eLIFE 2016; 5: e10147.

(2) Hentges DJ. The anaerobic microflora of the human body. Clin Infect Dis 1993; 16 (Suppl 4): S175–S180.

(3) Sanford JA, Gallo R. Functions of the skin microbiota in health and disease. Sem Immunol 2013; 25(5): 370–377.

(4) Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. Science 2005; 308(5728): 1635–1638.

(5) Dewhirst FE, Chen T, Izard J, et al. The human oral microbiome. J Bacteriol 2010; 192(19): 5002–5017.

(6) Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol 2011; 9(4):244–253.

(7) Zoetendal EG, Vaughan EE, DeVos WM. A microbial world within us. Molecular Microbiol 2006; 59(6): 1639–1650.

(8) Ogra PL. Mucosal microbiome and its impact on mucosal immune system in childhood. J Ped Infect Dis Immunol 2014; 26(1):77–84.

(9) Tannock GW. Normal microflora: an introduction to microbes inhabiting the human body. Amsterdam, The Netherlands: Springer, 1994.

(10) O'Toole PW and Jeffrey IB. Gut microbiota and aging. Science 2015; 350(6265): 1214–1215.

(11) Kostic AD, Howitt MR, Garrett WS. Exploring host-microbiota interactions in animal models and humans. Genes Dev 2013; 27(7): 701–718.

(12) Colombo BM, Scalvenzi T, Benlamara S, et al. Microbiota and mucosal immunity in amphibians. Front Immunol 2015; 6:111.

(13) Ogra PL. Mucosal immune system in neonatal period and early infancy. Pediatr Health. 2010; 4(6): 637–647.

(14) Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat Rev Immnol 2007; 7(5): 379–390.

(15) Kabat AM, Srinivasan N, Maloy KJ. Modulation of immune development and function by intestinal microbiota. Trends in Immunol 2014; 35(11): 507–517.

(16) Duffy LC, Zielezny MA, Riepenhoff-Talty M, et al. Reduction of virus shedding by B. bifidum in experimentally induced MRV infection: statistical application for ELISA. Digest Dis Sci 1994; 39(11): 2334–2340.

(17) Griffiths E, Duffy LC, Schanbacher FL, et al. In vitro growth responses of bifidobacteria and enteropathogens to bovine and human lactoferrin. Digest Dis Sci 2003; 48 (7): 1324–1332.

(18) Qiao H, Duffy LC, Griffiths EA, et al. Immune responses in rhesus rotavirus-challenged BALB/c mice treated with bifidobacteria and prebiotic supplements. Pediatr Res 2002; 51(6): 750—755.

(19) Clemente JC, Ursell LK, Parfrey LW, et al. The impact of the gut microbiota on human health: an integrative view. Cell 2012 148(6): 1258–1262.

(20) Ubeda C, Lipuma L, Gobourne A, et al. Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. J Exp Med 2012; 209(8): 1445–1456.

(21) Holmes E (2013) Mapping the consequences of metabolic interactions between host and microbiome on the brain. In: Heidt PJ, Bienenstock J, Rusch V (eds) The gut microbiome and the nervous system. Old Herborn University Symposium Series Monograph 26. Old Herborn Univ Foundation, Herborn-Dill, Germany, pp 49–59.

(22) Cryan JF (2013). Microbiota, stress and the brain. In: Heidt PJ, Bienenstock J, Rusch V (eds). The gut microbiome and the nervous system. Old Herborn University Symposium Series Monograph 26. Old Herborn Univ Foundation, Herborn-Dill, Germany, pp 27–37.

(23) DiMartino C, Basset C, Ogier A, et al. Distribution and phenotype of rotavirus-specific B cells induced during the antigen-driven primary response to 2/6 virus-like particles administered by the intrarectal and the intranasal routes. J Leukoc Biol 2007; 82(4): 821–828.

(24) Waffarn EE, Baumgarth N. Protective B cell response to flu – no fluke. J Immunol 2001; 186(7): 3823–3839.

(25) Valdez Y, Brown EM, Finlay BB. Evidence of the microbiota on vaccine effectiveness. Trends Immunol 2014; 35(11): 526–537.

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RANDOMIZED CLINICAL TRIAL: GUT MICROBIOME BIOMARKERS ARE ASSOCIATED WITH CLINICAL RESPONSE TO A LOW FODMAP DIET IN CHILDREN WITH IRRITABLE BOWEL SYNDROME

Chumpitazi BP, Cope JL, Hollister EB, Tsai CM, McMeans AR, Luna RA, et al. Aliment Pharmacol Ther. 2015; 42(4):418–427. doi:10.1111/apt.13286.

Irritable bowel syndrome (IBS) is a debilitating disorder prevalent in children, with approximately 20% of school-aged children experiencing abdominal symptoms related to IBS. However, there is no effective treatment for this condition. Recent evidence suggests a correlation between changes in gut microbiota and IBS, with several microbial patterns described for various subtypes of IBS. Probiotic interventions have been considered as a therapeutic option but have generated mixed results. Diet is another important factor for the modulation of gut microbiota and several diets have been shown to affect gut microbial content and diversity. A low FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) diet is a popular intervention to improve abdominal discomfort in IBS. Patients respond rapidly to this diet, as early as within 48 hours, and this response is durable most of the time. However, 25% of adult patients do not respond to this diet and little is known about the predictive factors for response in patients with IBS, although gut microbiota characteristics might have a role to play.

Investigators searched for gut microbiota signatures which could predict the response to a low FODMAP diet in children with IBS. Thirty-three children diagnosed with IBS according to ROME III criteria, were allowed to eat their regular diet for 7 days. Afterwards they were randomized to either low FODMAP or TACD (typical American childhood diet) for 48 hours. Following 5 days of wash-out period, they were crossed-over to the other diet for 48 hours.

Stool samples were obtained on the habitual diet phase and on the second day of each dietary intervention period and breath samples were collected hourly to measure hydrogen and methane levels. The primary endpoint was the number of daily abdominal pain episodes while the secondary endpoints were pain severity, composite gastrointestinal (GI) symptom score, and breath hydrogen-methane production; these results were compared with baseline microbiome data.

Abdominal pain improved and the total composite GI score decreased in patients in the low FODMAP group compared with patients in the TACD group. Breath hydrogen levels were lower with the low FODMAP diet while methane levels remained similar to baseline data. The investigators examined microbiome signatures in responders versus non-responders; different microbial Open Taxonomic Units (OTUs) were detected in responders versus non-responders. Sixty-three OTUs were accumulated in responders and four OTUs were enriched in non-responders. Responders to the low FODMAP diet had higher levels of saccharolytic metabolic capacity within the family Bacteroidaceae (e.g., Bacteroides), order Clostridiales (e.g., Ruminococcaceae, Dorea, and Faecalibacterium prausnitzii) and family Erysipilotrichaceae. However, non-responders had the genus Turicibacter from the family Turicibacteraceae.

Gene orthologs related to FODMAP carbohydrate metabolism and in particular the metabolism of wheat in FODMAP diet, including alpha-N-arabinofurasonidase, were found in responders but not in non-responders. There were no significant differences in alpha or beta diversity or in baseline dietary composition between responders and non-responders.

This is the first study to show that the response to a low FODMAP diet is directly related to the composition of the baseline endogenous microbiome. Mainly, the greater saccharolytic capacity of the microbiome was correlated with clinical response. The methodology applied to this study was robust and appropriate for the desired outcome; it represents a first step towards the development of personalized nutritional therapy for IBS based on microbiome composition and offers new therapeutic prospects for this debilitating disorder.

ESOPHAGEAL MICROBIOME IN EOSINOPHILIC ESOPHAGITIS

Harris JK, Fang R, Wagner BD, Choe HN, Kelly CJ, Schroeder S, et al. PLoS One. 2015; 10(5):e0128346. doi:10.1371/journal.pone.0128346.

Although eosinophilic esophagitis (EoE) is prevalent in developed countries, its etiology is not well-documented. Atopic responses are the mainstay of the pathogenesis of EoE and eosinophilic infiltration of the stratified epithelia of the esophagus is the histological hallmark. The microbiome has been studied in atopic diseases such as atopic dermatitis and bronchial asthma and correlations between certain microbiome patterns and atopic disorders have been established. In this study, the investigators aimed to identify specific microbial signatures in EoE, gastroesophageal reflux disease (GERD) and normal epithelium.

The population enrolled for this prospective study consisted of 70 children and adults and samples were collected using an Esophageal String Test (EST). Bacterial diversity and composition were determined by 16S rRNA gene amplification and pyrosequencing methods. Results were analyzed according to patient characteristics with four groups determined: EoE-untreated (active), EoE-treated (remission), GERD, and normal esophagus.

Bacterial load was higher in patients with EoE, regardless of the disease activity or treatment. When sequencing was performed at the phylum and genus level, four phyla were determined in the EoE groups (Bacteroidetes, Firmicutes, Fusobacteria and Proteobacteria). Patients with GERD showed an increase in Firmicutes and a decrease in Proteobacteria. Proton pump inhibitor (PPI) treatment in patients with GERD also increased the level of Proteobacteria. Interestingly, this PPI effect on microbiome was unique to patients with GERD. Patients with EoE using PPI had no apparent change in Proteobacteria levels or other genera. The comparison of untreated EoE with normal subjects identified that Haemophilus was the predominant bacteria. All subjects tested positive for Haemophilus but untreated patients with EoE had the highest amount of this bacterium. Furthermore, the microbiome composition was different between the Chicago center and the Aurora center, suggesting geographical variation.

This study showed that untreated patients with EoE have a different microbiome profile in terms of relative abundance of Haemophilus compared with treated patients with EoE, patients with GERD and healthy subjects. Epithelial eosinophilia in histological specimen did not influence the bacterial load, which was predominantly correlated to inflammation in untreated versus treated EoE patients. Patients with GERD treated with PPI had a profound predominance of Aggregatibacter and a decrease in Streptococcus (Firmicutes). This effect of PPIs was unique to patients with GERD. The selection of certain bacteria is PPI treatment might be influenced by proton pumps in bacteria, increased pH in gastric contents and esophageal lumen. Further research is needed to clarify this issue.

The difference between untreated versus treated EoE microbiome might be partly explained by anti-microbial properties of eosinophilic granules (defensins, granule proteins, extracellular DNA traps). There is a shift from Gram positive bacteria (Firmicutes) to Gram negative bacteria (Proteobacteria) in untreated EoE patients. Another possible confounding factor is diet, which is known to influence microbiome. Elimination diets in EoE patients and other therapies such as corticosteroid might change the microbiome profile. As a result, there is a substantial change in esophageal microbiome in patients with untreated EoE. The question remains whether this change in esophageal microbiome is, to some extent, driving disease progression or is simply a consequence of EoE; longitudinal studies designed to include larger cohorts are needed to answer this question.

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VASCULAR MICRORNA-204 IS REMOTELY GOVERNED BY THE MICROBIOME AND IMPAIRS ENDOTHELIUM-DEPENDENT VASORELAXATION BY DOWNREGULATING SIRTULIN[1]

Vikram A, Kim Y-R, Kumar S, Li Q, Kassan M, Jacobs JS, Irani K. Nat Commun. 2016; 7: 12565. doi: 10.1038/ncomms12565.

Atherosclerosis is one of the leading causes of morbidity and mortality worldwide. In addition to established risk factors, previous studies have reported a connection between atherosclerosis and gut microbiota. This study used a mouse model to investigate the role of vascular *microRNA-204 (miR-204)* on Sirtulin 1 lysine deacetylase (Sirt1), which is a key factor in nitric oxide (NO) synthesis in endothelium. Sirt1 is a class III histone deacetylase expressed in endothelial cells that stimulates endothelial NO synthase by deacetylation. Sirt 1 function is tightly regulated by microRNAs, namely *miR-217, miR-212* and *miR-204*. Of these microRNAs, *miR-204* has a unique feature in that its expression is also regulated via gut microbiota-related signals.

This study included two groups of animals: microbiota-free germ-free mice (GFM) and control pathogen-free mice (PFM). Of the 578 microRNAs analyzed, 15 were downregulated and 5 were upregulated in GFM compared with PFM. Overall microRNA expression and expression of microRNAs related to vascular endothelium did not differ between GFM and PFM.

In order to understand the effect of antibiotics on the expression of *miR-204,* mice were given antibiotics for 6 weeks. Mice on antibiotics had lower aortic *miR-204* expression than untreated mice. The beneficial effects of antibiotics were reversed after drug discontinuation. Total and endothelial Sirt1 was also upregulated in antibiotic-treated mice. Antibiotics stimulated gut microbiota-related expression of vascular NO and endothelium dependent vasorelaxation.

Diet is another important factor for gut microbiota composition. This study investigated the effects of a high-fat diet (HFD) on these parameters. After 8 weeks of HFD, the mice had upregulated *miR-204* expression and downregulated Sirt1 expression. Antibiotic administration during the last 6 weeks of HFD reversed these deleterious effects.

The molecular mechanism behind the effects of antibiotics and HFD on *miR-204* and Sirt1 expression was also investigated. Regulation of the transcription factor Stat3 expression via the gut microbiome is a key mechanism for *miR-204*-induced vascular changes. In this study, inhibition of Stat3 signaling resulted in stimulation of *miR-204* expression. Decreased levels of activated Stat3 were also observed in HFD mice. Antibiotic administration suppressed the microbiome and restored Stat3 signaling. This mechanism shows how the gut microbiome can influence endothelial function.

HFD-induced endothelial dysfunction is mediated by *miR-204*. When anti-*miR-204* was delivered to HFD mice, Sirt1 downregulation was prevented and vascular inflammation was suppressed.

Microbiome-related factors regulate *miR-204* expression. Antibiotics also reversed the effects of microbiome-related factors. In endothelial cells that were incubated with serum from antibiotic-treated mice, *miR-204* expression was downregulated and Sirt1 expression was upregulated.

In conclusion, these findings suggest that there is a «gut-vascular axis» that remotely regulates vascular endothelial function via the microbiome.

PROTON PUMP INHIBITORS ALTER SPECIFIC TAXA IN THE HUMAN GASTROINTESTINAL MICROBIOME: A CROSSOVER TRIAL

Freedberg DE, Toussaint NC, Chen SP, Ratner AJ, Whittier S, Wang TC,et al. Gastroenterology. 2015; 149:883–885. doi: 10.1053/j.gastro.2015.06.043.

Clostridium difficile infection (CDI) is one of the most debilitating conditions among hospitalized elderly patients in developed countries, including the USA. Proton pump inhibitors (PPIs) are widely used in hospitalized patients and patients in the intensive care unit. Preliminary reports have suggested that PPIs have deleterious effects on the microbiota that predispose the host gut microbiome to CDI. This study published in *Gastroenterology* aimed to address this question.

This study was an open-label, crossover trial, conducted in 20 healthy volunteers. The volunteers provided two baseline fecal samples (to account for daily variation of the microbiome), collected 4 weeks apart (weeks 0 and 4). The volunteers then received PPI therapy (40 mg omeprazole twice daily) for 4 weeks, and provided a third fecal sample at the end of the week 8. Six volunteers were randomized to continue PPI therapy for an additional 4 weeks (from week 8 to 12), after which the final fecal samples from this prolonged-therapy group were obtained at week 12. Exclusion criteria included antibiotic consumption in the previous year, and *C. difficile* toxin-positive patients. The primary outcome was fecal microbial diversity, defined as the difference in the Shannon's index of diversity for each individual between the 4-week baseline period and the 4-week PPI treatment period. However, the study also focused on the taxa related to CDI-prone bacterial populations using prespecified taxa of interest (based on previous studies).

After 4 weeks of PPI therapy, there were no intra-individual changes in gut microbiota diversity. Furthermore, there were no differences in diversity in patients on prolonged (8 weeks) PPI therapy.

Apart from bacterial diversity, the study found that 4 weeks of PPI therapy significantly increased Enterococcaceae and Streptococcaceae taxa, which are often associated with a CDI-prone milieu in the gut. Enterococci levels are low in the healthy gut microbiome, but may be rapidly selected for after broad-spectrum antibiotic usage. Streptococci, which are predominantly found in the upper gastrointestinal tract, showed >10-fold increases after PPI treatment in this study. Streptococci have also been associated with CDI in previous studies.

Across 97 bacterial taxa present in all samples, there was a 44% median decrease in Clostridiaceae taxa after 4 weeks of PPI therapy. There were no further changes in the microbiome at week 12.

Secondary bile acids, which are produced by bacteria, play an important role in the spore germination process of *C. difficile*. Many studies have found that secondary bile acid composition in the colon is one of the most important predictors of CDI. In cirrhotic patients who were administered PPIs, secondary bile acid production was decreased and Streptococci increased. However, in the current study, there were no changes in secondary bile acids after PPI therapy.

Further metagenomic analysis revealed significant increases in pathways corresponding to genes responsible for bacterial invasion of epithelial cells and the renin-angiotensin system.

In conclusion, this pilot study indicates that PPIs alter microbiome composition and microbial functions. Some bacterial taxa are increased and bacterial genes show different expression levels. Although 4 weeks of PPI therapy (without antibiotics) may not directly predispose an individual to CDI, it may lower gut colonization resistance against *C. difficile*.

REPORT OF THE 49TH ANNUAL MEETING OF THE EUROPEAN SOCIETY OF PAEDIATRIC GASTROENTEROLOGY, HEPATOLOGY AND NUTRITION

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This year the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Annual Meeting was held in Athens, Greece, May 25th–28th, 2016. The congress presented a very attractive scientific program, focusing on the most important issues around pediatric gastroenterology, hepatology and nutrition. The program was preceded by a comprehensive set of postgraduate courses for physicians, for allied health care professionals, as well as a course focusing on pediatric inflammatory bowel disease. Participants had also the opportunity to develop their endoscopy skills at the Learning Zone.

This year, many participants presented interesting new results on the composition of the intestinal microbiota in neonates and children and how it changes under different pathological conditions. The therapeutic and preventive effects of probiotics on different diseases were also displayed in several presentations. Therefore, aspects of these two main concepts discussed at the ESPGHAN 49th Annual Meeting will be conveyed in this report.

Changes of intestinal microbiota composition in different pathological conditions

Amit Assa (Petach Tikva, Israel) studied the mucosa-associated ileal microbiota in new-onset pediatric Crohn's disease. Numerous operational taxonomic units associated with *Faecalibacterium prausnitzii* species were increased in patients with Crohn's disease. This finding challenges the view that this bacterium has a protective role in Crohn's disease.

Tim de Meij (Amsterdam, The Netherlands) gave a fascinating presentation on short bowel syndrome and how intestinal microbiological analysis may be used to detect necrotizing enterocolitis (NEC) and late onset sepsis early. Fecal samples were collected daily from birth to day 28 in 385 infants born at gestational age <30 weeks. In infants who developed NEC, *Citrobacter koseri* and *Clostridium perfringens* were detected in the days prior to NEC. Patients with sepsis showed a significantly lower density of Proteobacteria than those with NEC. These observations suggest that microbiota profiling might allow the selection of a subgroup of neonates who are at increased risk of developing NEC in early stages.

Jan Knol (Wageningen, The Netherlands) examined changes in the composition of the intestinal microbiota after short (<3 days) and long (>5 days) antibiotic treatment courses in late-preterm and term infants. The abundance of *Bifidobacterium* was significantly decreased immediately after treatment with antibiotic therapy. The number of *Enterococcus* increased after short or long treatment. The abundance of *Bifidobacterium* was restored after six weeks in patients receiving a short antibiotic course, while this was not the case in patients receiving a long antibiotic course.

Ravider Nagpal (Tokyo, Japan) investigated the differences in intestinal microbiota of infants born by vaginal delivery versus those born by cesarean-section. The colonization rate of alpha-toxigenic *C. perfringens* was significantly higher in cesarean-born infants at 6 months, but breast-fed infants were significantly less often colonized with *C. perfringens* and *C. difficile* compared with formula-fed infants.

Ravidel Nagpal and colleagues also presented a study in which the composition of intestinal microbiota of healthy young adults who were delivered by cesarean-section was compared with the intestinal microbiota of those who were born by vaginal delivery. The levels of *Bacteroides fragilis* group and *Lactobacillus sakei* subgroup were higher in vaginally-delivered subjects compared to those born by cesarean section. This study suggests that differences in gut microbiota as a result of birth by cesarean section may persist even into adulthood. Disturbed intestinal colonization after cesarean section delivery is associated with increased risk of both allergic and autoimmune disease during the life.

Konstantinos Gerasimidis (Glasgow, UK) examined the fermentation capacity of the gut microbiota in children with inflammatory bowel disease (IBD). Fresh fecal samples were collected from patients with IBD in clinical remission and healthy children; *in vitro* batch culture fermentations were carried out for eight carbohydrate/fibers. It was revealed that the microbiota of patients with IBD had a lower capacity to break down fiber than the microbiota of healthy children and that both butyrate and acetate productions were reduced in patients with IBD.

Lorella Paparo and colleagues (Naples, Italy) investigated the gut microbiota in children with autism spectrum disorders (ASD). All ASD children showed significant alterations in gut flora compared with healthy children at both species and phyla levels (Actinobacteria, Bacteriodetes, Firmicutes and Proteobacteria) by UniFrac analysis. Children with ASD also had higher levels of fecal butyrate than healthy children.

Azelea Rushd A and colleagues from Imperial College London assessed the effects of sample size and bowel preparation on the colonic microbiome of children. They compared the composition of the microbiome from samples collected from rectal biopsies, rectal swabs and fecal samples collected from the most distal colonic location during colonoscopy. It was shown that a clinically relevant description of the mucosal microbial community could be obtained from the distal colonic content, which was less invasive and was not disturbed by bowel preparation for colonoscopy. Rectal swabs were not closely representative of the mucosa-associated colonic microbiome.

In a piglet experimental model, Isabelle Le Huerou-Luron and colleagues (Paris, France) demonstrated that infant formula supplemented with a mixture of cow milk and vegetable lipid incorporated to milk fat globule membrane led to an intestinal microbiome composition closer to that of suckling piglets, than when piglets were fed only vegetable milk.

Modification of intestinal microbiome with probiotics, prebiotics and synbiotics in different pathological conditions

Tamás Decsi and colleagues (Pécs, Hungary) investigated the effect of prebiotic inulin-type fructans on health parameters and the composition of intestinal microbiota in children aged 3 to 6 years in a randomized, double-blind, placebo-controlled study. A total of 110 children received inulin-type fructans and 109 received placebo. *Bifidobacteria* counts were significantly higher in the prebiotic group than in the control group after 24 weeks, while the *Lactobacillus* counts decreased. In the prebiotic group, the stool became softer within the normal range, the number of febrile episodes requiring a consultation with a physician and sinusitis events were significantly lower than in the placebo group.

Otuzbir A and colleagues (Bursa, Turkey) studied the therapeutic effect of a synbiotic product containing *L. acidophilus, L. casei, Bifidobacterium lactis* in a total of $7x10^9$ colony-forming unit (CFU) and 100 mg dandelion inulin in functional abdominal pain. After the 8-week treatment period, no change in complete remission was observed between the synbiotic and the placebo group. Partial resolution of symptoms was slightly higher in the synbiotic group (80 vs 63 %, p=0,05).

Bastürk A and colleagues (Antalya, Turkey) conducted a randomized, double-blind, controlled study to assess the efficacy of synbiotic, probiotic and prebiotic treatments in children with irritable bowel syndrome (IBS). The probiotic treatment consisted of $5x10^9$ CFU of *B. lactis* B94, the prebiotic treatment of 900 mg inulin, and the synbiotic treatment was a combination of both in the same doses. The proportion of patients with full recovery was significantly higher in the synbiotic group than in the prebiotic group (39.1% vs 12.5%); no significant improvement was observed in the prebiotic group.

Oleg Jadresin and colleagues (Zagreb, Croatia) studied the effect of 108 CFU *L. reuteri* DSM17938 in children with functional abdominal pain or IBS; they observed a reduction in pain intensity and a significant increase in the number of days without pain.

Lorella Paparo L and colleagues (Naples, Italy) observed that, *in vitro,* fermented rice containing *L. paracasei* CA L74 decreased the production of interleukin (IL)-4 and IL-5 by peripheral blood mononuclear cells (PBMCs) isolated from children with cow milk allergy when these cells were stimulated with bovine beta-lactoglobulin. PBMCs also produced more IL-10 and interferon-gamma. These results suggest that this probiotic is able to modulate the Th1/Th2 response in children with cow milk allergy.

The 49th ESPGHAN annual meeting, held in Athens, was very successful; over 4000 delegates attended, coming from Europe as well as other continents. The congress offered a great platform to meet colleagues from around the world who study different aspects of pediatric gastroenterology, hepatology and nutrition. Bringing together international leaders provides a unique opportunity to discuss the latest advances in this area of research and form networks for collaboration across different countries within Europe but also with the rest of the world.

THE GUT MICROBIOTA IN DDW 2016

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Digestive Disease Week (DDW) 2016 took place on May 22nd–24th in San Diego. Gut microbiota and microbiome studies were one of a prevalent topic of the congress. Many oral presentations and posters have shown interesting results regarding the composition of the intestinal microbiota and how it changes under various pathological conditions and the effects of fecal transplantation and probiotics in different gastrointestinal (GI) diseases and obesity.

Changes of intestinal microbiota composition in different pathological conditions

Alain P. Gobert (Nashville, Tennessee, USA) and colleagues from France have shown that the intestinal microbiota of patients with irritable bowel syndrome (IBS) attenuates dextran sulfate sodium (DSS)-induced colitis in rats. Using human microbiota-associated Germ-free Sprague Dawley rats, they reported that microbial imbalance initiates perturbation of the host intestinal mucosal immune response during an experimental colitis versus sham animals. This study indicates that the gut microbiota of patients with IBS may exhibit anti-inflammatory properties.

Data presented by Francesca Romana Ponziani (Rome, Italy) suggested that small intestinal bacterial overgrowth (SIBO) is linked to vascular disease via vitamin K2-dependent mechanisms. While the source of vitamin K2 in Western populations is gut bacteria, its daily intake was lower in patients with SIBO. This may explain how dysbiosis could contribute to the risk of vascular calcifications and increased arterial stiffness.

Williams Turpin (Toronto, Ontario, Canada) investigated the association between demographic and environmental factors with fecal microbiota composition in a population of 840 healthy Caucasian first degree relatives (FDR) of patients with Crohn's disease (CD). The V4 hypervariable regions of 16S rRNA were sequenced from bacterial DNA extracted from stools. In additions, 23 patients with CD, 86 with inflammatory bowel disease (IBD) and 14 with ulcerative colitis (UC) were included. The effects the genetic risk score (GRS), the environmental, and demographic factors had on microbiota were calculated. Although no significant association between any of the 123 IBD risk-single nucleotide polymorphisms and microbiota were found, 25 taxa were significantly associated with environmental factors in a model corrected for age and gender that used GRS as a covariate.

Floris Imhann (Groningen, Netherlands) presented data from an integrated single-center case-control analysis of the luminal gut microbiome, the host genome and the clinical phenotypes of IBD. The aim was to evaluate individual differences in the complex interaction between the host genome and gut microbiome that could account for the heterogeneous presentation of IBD. The microbiome composition of the stool samples from 313 patients with IBD and 582 healthy subjects was assessed by tag-sequencing 16S rRNA gene. Large differences in the gut microbiome of colonic disease (UC and colonic CD) versus ileal disease were shown. This was the first report of genetic risk variants associated with IBD influencing the gut microbiome in healthy individuals. In patients with IBD, a decrease of acetate-to-butyrate converters (*Roseburia* spp.) was found. The authors hypothesized that the onset of IBD could be explained by impaired bacterial handling by gut immune system with a direct effect on the gut microbiota leading to a pro-inflammatory response of the gut microbiome. Genetic IBD risk appears to be related to the *Roseburia* genus in healthy individuals.

Findings presented by Allison Agus (Clermont-Ferrand, France) were fascinating as they showed that a Western diet induces a shift in composition of microbiota and in turn increased susceptibility to adherent-invasive *Escherishia coli* infection, creating a low-grade inflammation in the gut.

Robert Fedorak (Edmonton, Alberta, Canada) examined the effects of a high-sugar diet on colonic gene expression and microbial composition in patients with IBD. It was found that host susceptibility to colitis was increased by promoting the growth of an inflammatory microbiota and reducing the expression of genes related to barrier function. This suggests that a high sugar diet may also increase the risk of developing colorectal cancer through enhancing inflammation.

Results from an experimental study presented by Benoit Chassaing (Atlanta, Georgia, USA) confirmed that dietary emulsifiers directly impact the human gut microbiota by increasing its pro-inflammatory potential.

James L. Alexander (London, United Kingdom) examined the microbiome-metabolome interactions in colorectal cancer using mass spectrometry imaging in a prospective, observational multicenter study in a single cohort of patients undergoing surgery for colorectal tumors. This analysis revealed that inter-individual variation significantly influence the mucosal expression of pathobionts linked to colorectal cancer; therefore, mutualistic community metabolism within geographically discrete regions of tumors is likely to be of functional importance to the etiology of cancer.

Evelien F. de Groot (Amsterdam, Netherlands) investigated the use of intestinal microbiota as a tool for early detection of necrotizing enterocolitis (NEC). It was shown that the composition of microbiota in patients with NEC differed from that found in patients with sepsis or healthy subjects as early as 5 days prior to onset of NEC. This data confirmed the crucial role of the microbiome in the development of NEC. Detailed understanding of specific microbial shifts may lead to the development of targeted, individualized preventive strategies (use of prebiotics, probiotics or selective antibiotics) to decrease mortality and morbidity.

Microbiome determinants of Clostridium difficile infection

Sahil Khanna (Rochester, Minnesota, USA) evaluated pre-treatment stool samples and clinical outcomes of 88 patients with a first episode of *Clostridium difficile* infection (CDI). The V4 region of the 16S rRNA gene was used for gut microbiome profiling. Patients with recurrent CDI versus those with non-recurrent CDI had an increase in Veillonella, Enterobacteriaceae, Streptococcus, Parabacteroides and Lachnospiraceae species in fecal samples. The author recommended the detection of those microbiome signatures in pre-treatment stool as predictors of recurrent CDI after success of initial treatment.

Monika Fischer (Indianapolis, Indiana, USA) studied the long-term risk of CDI recurrence after a successful fecal microbiota transplant (FMT) with or without exposure to antibiotics. A questionnaire was used to collect information from 265 patients (152 patients with complete follow-up) about use and type of any non-CDI antibiotics, concomitant prophylactic anti-CDI antibiotics, probiotic and CDI recurrence after FMT. The overall CDI recurrence rate was 10.5% and of these 62.5% were related to non-CDI antibiotic.

Hon Wai Koon (Los Angeles, California, USA) showed that Saccharomyces boulardii CNCM I-745 can protect from CDI caused by hypervirulent *C. difficile* strains through a mechanism involving inhibition of the cytotoxic effects of *C. difficile* toxins.

Aiming to understand their role in pathogenesis of CDI, Jessica R. Allegretti (Cambridge, Massachusetts, USA) compared bile salt profiles of patients with CDI with those of healthy subjects. Sequencing of 16S rRNA gene and bile salt metabolomic analysis were performed and a total of 60 patients (20 with a first episode of CDI, 19 with recurrent CDI, and 21 healthy subjects) were enrolled. It was shown that secondary bile acids (which have a protective effect) were significantly elevated in healthy subjects compared with both CDI groups in stools as well as in blood. In contrast, primary bile acids (inductors of germination) were elevated in the recurrent CDI group only. The authors concluded that plasma deoxycholate was a strong predictor of disease state and may be used as a marker of recurrence.

Studies of probiotics efficacy

Morris Gordon (Preston, United Kingdom) presented a Cochrane systematic review of the use of probiotics in the management of functional abdominal pain in children. A meta-analysis of seven studies (N=541) found a statistical significant reduction in the severity of pain in patients receiving probiotics compared with those receiving placebo (mean difference –0.32; 95% confidence interval [CI] –0.38 to –0.25). Another meta-analysis of 4 studies (N=440) found a statistical significant difference in patients reaching treatment success in favor of probiotics compared with placebo (OR 1.80; 95% CI 1.20 to 2.69). Lastly, a meta-analysis of five studies (N=385) found no statistically significant difference in the number of adverse events reported between patients receiving probiotics and those receiving placebo (OR 0.00; 95% CI –0.07 to 0.06). The authors concluded that the evidence base was of moderate quality and relatively small but warranted further research to investigate the long-term impact of probiotic therapy.

Nicole T. Shen (New York, USA) presented a systematic review and meta-analysis of the use of probiotics for the prevention of CDI in hospitalized adults receiving antibiotics. A total of 18 studies (N=6129) were included in the analysis. The incidence of CDI in the probiotic cohort was significantly less than that in the control cohort (1.6% vs 3.9%, p=0.003), with an absolute risk reduction of 2.3% and a number needed to treat of 43. Cumulative meta-analysis showed robust efficacy of probiotics in the prevention of CDI, with a relative risk of 0.38 (95% CI 0.27 to 0.54; p≤0.000; I2 = 7.1%). Of the nine different probiotics used, *L. acidophilus* and *L. casei* were the most effective, with a relative risk of 0.17 (95% CI 0.08 to 0.37; p≤0.000; I2 = 0%). Differences in formulation (milk versus capsule) did not reach statistical significance and unadjusted meta-regression did not demonstrate a dose response. There was no report of probiotic sepsis. This data strongly suggests that the use of probiotics significantly reduces the risk of CDI in hospitalized patients taking antibiotics and although further studies are no longer needed to establish efficacy, optimal doses and strains remain to be determined.

Another study also presented by Nicole T. Shen and al highlighted the use of probiotics as a cost-effective strategy to prevent CDI in hospitalized adults aged 65–84 and >85 when undertaking high probiotic efficacy , but not in other age cohorts or with low efficacy assumptions . Results were sensitive to probiotic cost and baseline risk of CDI.

SAN DIEGO – Patients with hepatitis C virus (HCV) infections had distinct duodenal mucosal microbiomes and greater intestinal permeability, compared with healthy controls and patients with other chronic liver diseases, Dr. Ashok Raj reported.

The findings might one day lead to therapies that aim to restore or normalize the microbiomes of patients with HCV, Dr. Raj said in an interview at the annual Digestive Disease Week.

Chronic liver disease (CLD) has been linked to dysbiosis, or abnormal shifts of the microbiome. But most studies have focused on fecal specimens, and «recent evidence suggests that the mucosal microbiota differ from fecal microbiota,» said Dr. Raj, a gastroenterologist and hepatologist at Princess Alexandra Hospital in Brisbane, Australia, and a PhD candidate at the University of Queensland at Brisbane.

«The small-intestinal mucosal microbiota are of particular interest to us,» Dr. Raj explained. «Anatomically, all the blood from this region of the gut drains into the portal vein and flows directly to the liver. Because of small-intestinal permeability, either bacteria or their products could travel to the liver and contribute to disease. But very little is known about this microbiota in CLD.»

Therefore, Dr. Raj and his associates sequenced bacterial DNA from mucosal biopsies of the second part of the duodenum from 38 prospectively recruited endoscopy patients with CLD and 13 healthy controls. The researchers also evaluated dietary habits, intestinal permeability, hepatic stiffness based on transient elastography, and the presence of metabolic syndrome, as measured by the International Diabetes Federation/American Heart Association/National Heart, Lung, and Blood Institute 2009 Consensus criteria. The CLD group included 28 men and 10 women aged 36-82 years, including 16 patients with HCV, 10 patients with nonalcoholic fatty liver disease, 7 patients with fatty liver disease, 3 patients with autoimmune hepatitis, and 2 patients with hepatitis B virus infection. The controls were between 24 and 73 years old, and 70% were women.

Sequencing of bacteria DNA revealed significant differences between patients and controls, particularly among patients with HCV, Dr. Raj said. Patients with HCV not only had significantly less microbial diversity ($p<0.02$), but the overall changes in their microbiota were significant enough for them to cluster separately from controls and from patients with other types of CLD ($p<0.01$ for both comparisons). Furthermore, patients with HCV had significantly greater small-intestinal permeability (mean ± SD log lactulose to rhamnose ratio, 1.57 ± 0.27) than controls (1.21 ± 0.25; $p<0.01$) or patients with other CLDs (1.24 ± 0.39; $p=0.01$).

«Additionally, for patients with HCV, dietary fat intake showed a moderately strong positive correlation with intestinal permeability,» Dr. Raj said ($r=0.58$; $p=0.03$). «These findings are in keeping with animal models, which have shown that dietary fat can change the microbiota and also increase intestinal permeability.» However, the multivariate analysis found no links between microbial characteristics and hepatic stiffness or metabolic syndrome – perhaps because most patients were «at the cirrhotic end of the spectrum, reflecting their indication for endoscopy,» or because «these relationships are subtler and require larger sample numbers,» he said.

«Patients with HCV may have a unique small-intestinal microbiome,» Dr. Raj concluded. «These patients had higher intestinal permeability, and it is possible that the microbiota have a part to play in that.» Exactly how microbiota and gut permeability contribute to disease remains unclear, but pathology in the small intestine could help explain some features of the HCV trajectory, such as extrahepatic manifestations or variations in disease progression, he added. «Future studies may lead to targeting the small-intestinal gut microbiome to modulate and even treat HCV.»

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BIOTASCOPE

Translational Science in Microbiota

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