Microbiome Analysis in the Esophagus

See "Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome," by Yang L, Lu X, Nossa CW, et al, on page ●●.

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Microbial Diversity Within the Human Body. The human body is estimated to contain 10^{13} to 10^{14} bacteria, or 10 times more bacteria than body cells, and at least 100 times more microbial than human genes.[1] The term superorganism has been used to reflect this composite nature of the human body,[2] and the word microbiome is applied to both the totality of bacteria in the entire human body (or a defined niche), or, in a narrower sense, for the combined genomes of these indigenous microbes. The human microbiome has been shaped by a long coevolution with its host,[3] like some individual microbes, some of which have been shown to reflect human history in surprising detail (eg, the gastric pathogen, *Helicobacter pylori*[4,5]).

In the last few years, a fast-growing body of evidence has accumulated, demonstrating that these microbial communities play an important role in human physiology. In turn, many diseases, in particular those of the gastrointestinal tract, are associated with changes in composition and diversity of these microbial communities. Only a minority of the bacteria that comprise the human microbiome can be cultured and classified by conventional microbiological methods. However, culture-independent methods have been used increasingly to characterize the diversity of the complex microbial communities that can be sampled from different sites of the body in healthy individuals and in patients with diverse pathologic conditions. The most widely applied approach makes use of the fact that all bacteria possess 1 or multiple copies of a gene (16S rDNA) that codes for the 16S ribosomal RNA. Some regions of this gene are so conserved that almost all eubacterial and archaeal 16S rDNA genes can be amplified with very few sets of broad-range polymerase chain reaction primers. Other regions between these conserved regions vary between species, and these variable sequences have been shown to provide valuable information about the taxonomic position of the respective microbe. It is this combination of highly conserved and highly variable sequences in a relatively short part of the bacterial chromosome (the length of the 16S rDNA gene is ~1,550 base pairs) that has made 16S rDNA sequencing the mainstay of bacterial diversity research.[6]

Recent improvements in sequencing technology, such as the development of massively parallel pyrosequencing,[7] have made it possible to even sequence all DNA extracted from a sample (the metagenome, which includes human DNA as well as bacterial, fungal, and viral DNA). However, metagenome sequencing requires extensive sequencing capacity, and the bioinformatic analysis of such datasets remains extremely challenging, so that 16S rDNA sequencing approach remains a practical approach to the study of bacterial diversity in human material. A special issue of GASTROENTEROLOGY published in May 2009 was devoted to the topic "Intestinal Microbes in Health and Disease" and contains several review articles covering the gastrointestinal microbiome as well as therapeutic perspectives arising from microbiome research.[8,9]

The Microbiome of the Distal Esophagus. In this issue of Gastroenterology, Yang et al[10] report the characterization of the microbiome of the distal esophagus in healthy subjects as well as patients with esophagitis or Barrett's esophagus. The 16S rDNA sequencing was used to characterize the bacterial diversity in biopsy samples taken from the distal esophagus of 34 individuals with either normal mucosa, esophagitis, or Barrett's esophagus (intestinal metaplasia). Approximately 200 partial 16S rDNA sequences were analyzed per sample. Although the number of sequences obtained per biopsy is certainly too low to provide a detailed picture of the microbiome that comprises many species with very low abundance, the authors succeed in demonstrating convincingly that the composition of the esophageal microbiome differs significantly between the healthy subjects and those with either esophagitis or Barrett's esophagus. The samples from healthy subjects were dominated by streptococci. On average, 78% of the sequences from healthy esophageal mucosa were categorized to belong to streptococcal species, and the numbers of all other species were so low that a meaningful interpretation of their quantitative contribution seems to be impossible. The authors use the term type I microbiome for this composition of the esophageal bacterial flora. By contrast, the microbiome in the samples from both groups of diseased tissue was overall more diverse, with a lower proportion of streptococci (29%) plus taxa related to streptococci (eg, *Gemella*, 8%), and significant numbers (>3%) of sequences that could be assigned to other, mostly Gram-negative taxa, such as *Prevotella*, *Bacteroides*, *Haemophilus*, and *Veillonella* (type II microbiome). The authors show that the

Table 1. Selected Studies That Used High-Throughput Culture-Independent Methods to Characterize the Microbiota of Sections of the Gastrointestinal Tract in Healthy Individuals and Patients With Gastrointestinal Disease

Sampled populations (*n*)	Material/site	Sequencing approach	Method	Reference
Human subjects				
Healthy individuals (3)	Colon mucosal biopsies, feces	16S rDNA	Sanger	18
Healthy individuals (2)	Feces	Metagenome	Sanger	2
Lean and obese female twins and their mothers (154)	Feces	16S rDNA, Metagenome	Sanger, 454 pyrosequencing	19
Patients with irritable bowel syndrome and controls (27)	Feces	16S rDNA	Sanger	12
Patients with ulcerative colitis and Crohn's disease and controls (190)	Tissue samples	16S rDNA	Sanger	15
Helicobacter pylori-positive and -negative individuals (23)	Gastric biopsies	16S rDNA	Sanger	13
Healthy individuals, patients with esophagitis and BE (34)	Biopsies from distal esophagus	16S rDNA	Sanger	10
Animal models				
Normal and obese mice (5)	caecum	Metagenome	Sanger, 454 pyrosequencing	20
Macaques with diverse pathologic conditions, including SIV infection and colitis (100)	Colonic content, tissue samples	16S rDNA	454 pyrosequencing	21

associations of these 2 types of microbiome with healthy (type I microbiome) and diseased esophagus (type II microbiome) are statistically significant. However, owing to its design, the study does not answer the question whether the presence of type II flora (or the absence of type I bacteria) plays a causal role in the pathogenesis of esophageal inflammation. The same conditions (in particular, acid reflux) that favor esophageal inflammation might suppress type I flora and favor colonization by type II flora. Alternatively, the inflamed mucosa might provide a more hospitable habitat than healthy epithelium for microaerophilic/anaerobic bacteria comprising the type II flora. These alternative hypotheses will have to be addressed by future studies, which should be conducted with a prospective design and involve a finer characterization of the microbiomes. It seems nevertheless likely that the profound change of the composition of the microbial community in the esophagus might influence epithelial function. For example, the diverse type II community with its larger content of Gram-negative bacteria might engage innate immune functions of the epithelial cells in a different way than the type I flora, owing to the release of a larger spectrum of microbial components stimulating pattern receptors (eg, Toll-like receptors). It should also be noted that the type II microbiome contained significant numbers of potential pathogens, such as *Campylobacter* species, and a significantly higher percentage of Gram-negative bacteria, whose relevance in the maintenance of inflammation remains to be elucidated. The change from microbiome type I to type II might thus prove to be an important step in the pathogenesis of esophageal tumorigenesis, and represent a biologically more plausible microbial component in this disease than the absence of *H pylori* from the stomach which has been reported to be associated with an increased risk of esophageal adenocarcinoma.[11]

Associations Between Microbiome Composition and Other Gastrointestinal Diseases. The analysis by Yang et al is an important addition to the still rather small group of studies that have characterized the gastrointestinal microbiome in well defined groups of individuals by culture-independent methods (Table 1). These include a comparison between the fecal microbial communities of healthy subjects and patients with irritable bowel syndrome, a study of the gastric microbiome in *H pylori*-positive and -negative individuals, and several studies of the microbiome of healthy persons versus patients with inflammatory bowel diseases[12–14] (see also Sartor[15] for an extensive and recent review). Most of these studies have also shown that the microbiomes of healthy individuals differ from those with specific gastrointestinal diseases, although none of them has yet been able to establish a functional or causal connection between these changes and the disease itself. Several recent review articles[9,15–17] provide more detailed coverage of this area than is possible in the context of this editorial.

Perspective. Massive efforts are currently invested into the field of human microbiome research. The Human Microbiome Project is part of the Roadmap for Medical Research (http://nihroadmap.nih.gov/hmp/) formulated by the US National Institutes of Health; other funding agencies have launched similar initiatives. However, in addition to massive sequencing capacity and expertise in the fields of genomics and bioinformatics, a large amount of clinical knowledge, hypothesis generation, and careful selection of patients and control individuals are essential to generate knowledge that will stand the test of time and ultimately lead to clinical

applications. Such future applications might be able to adjust compartments of the microbiome by deletion of harmful bacteria or addition of selected beneficial microbes. On the basis of individual "microbiome fingerprints," enhancements of the intestinal microbiome might help to shape the innate and adaptive immune responses, to promote tolerance development to nutrition and harmless agents, balance immune homeostasis, and increase human metabolic properties and versatility, contributing to the treatment of many different gastrointestinal conditions.

SEBASTIAN SUERBAUM
MHH Hannover Medical School
Institute of Medical Microbiology and Hospital Epidemiology
Hannover, Germany

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Reprint requests
Address requests for reprints to: Sebastian Suerbaum, MD, Professor and Director, Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany. e-mail: suerbaum.sebastian@mh-hannover.de; fax: +49 511 5324355.

Conflicts of interest
The author declares no conflicts.

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