主論文の要旨

Characterization of bacterial biota in the distal esophagus of Japanese patients with reflux esophagitis and Barrett's esophagus

（日本人の逆流性食道炎患者および
バレット食道患者における下部食道細菌叢の特徴）

名古屋大学大学院医学系研究科 分子総合医学専攻
病態内科学講座　消化器内科学分野
（指導：後藤 秀実　教授）
劉 寧

Background

In western countries, where gastroesophageal reflux disease (GERD) has long been common, the incidence of esophageal adenocarcinoma has increased progressively since the 1970s. Persistent GERD can lead to Barrett's esophagus. The incidence of GERD has recently also increased in Asian countries, particularly in Japan. Generally, the cause of esophageal diseases is still speculative. Host genetics may play a key role, but environmental factors are also likely involved. Colonizing bacteria in all parts of the human digestive tract, from the oral cavity to the anus, are essential to human survival. The digestive microbiota is a diverse and dynamic system which has developed a synergistic relationship with its host. Moreover, it also plays a crucial role in the development of the host's innate and adaptive immune system for the maintenance of a normal physiological environment.

The classical method of bacterial culture excludes a large number of unculturable bacteria, and also misrepresents the abundance of some species due to culture condition-related selection. To overcome these drawbacks, culture-independent methods have been developed, the most common of which involves the amplification and analysis of the 16S rDNA gene in a microbiome, on the basis that this gene contains highly conserved regions for the identification of individual species. We hypothesized that increased knowledge of bacterial communities in the distal esophagus would assist our understanding of the role of bacteria in diseases at this site.

Here, to better understand the role of bacteria in diseases of the distal esophagus, we examined bacterial composition at the 16S rDNA gene site in subjects with a normal esophagus, reflux esophagitis, or Barrett's esophagus using 16S rDNA gene-based culture-independent techniques.

Methods

Two biopsy specimens were obtained from the distal esophagus at 1 cm above the gastroesophageal junction under endoscopic examination in 18 patients (6 each with normal esophagus, reflux esophagitis, and Barrett's esophagus) (Table 1). And used for histological examination and DNA extraction. Fragments of 16S rDNA genes were amplified by PCR using general bacterial primers, and bacterial populations were examined. A third biopsy specimen was taken from the patients with Barrett's esophagus to histologically confirm the replacement of squamous epithelium with columnar epithelium in the distal esophagus.

Results

Quantification of bacterial populations

Measurement of total bacterial load in mucosal biopsy samples from the distal esophagus in control subjects and patients with reflux esophagitis and Barrett's esophagus showed high variability among samples, but no significant difference in the total amount of bacterial DNA

between the three groups ($p > 0.1$) (Figure 1).

Distribution of clones at the phylum and genus levels

Examination of bacterial populations in the distal esophagus by universal 16S rDNA PCR in biopsy samples from 18 subjects revealed an average of about 350 clones in each of the 18 subjects. Of these, 40 were randomly selected and sequenced. To characterize bacterial populations in normal esophagus, we analyzed bacterial flora from 6 subjects. Two hundred and forty clones (40 clones from each subject) yielded 147 16S rDNA sequences, all of which were classified into 4 phyla. *Proteobacteria* was the most prevalent phylum represented in normal subjects, accounting 49% of clones, followed by *Firmicutes* (40%),*Bacteroidetes* (8%), and *Actinobacteria* (3%) (Table 2). Members of 11 genera (≥3%) were observed, including Streptococcus (21%), *Klebsiella* (10%), *Gemella* (6%), *Eubacterium* (5%), *Citrobacter* (4%), *Granulicatella* (4%), *Haemophilus* (4%), *Helicobacter* (4%), *Escherichia* (4%),*Bulleidia* (3%), and *Prevotella* (3%) (Figure 2A). To characterize bacterial populations in reflux esophagitis, we analyzed bacterial flora from six patients with reflux esophagitis. Two hundred and forty (40 clones from each subject) clones yielded 139 16S rDNA sequences, all of which were classified into 6 phyla. *Proteobacteria* was the most prevalent phylum of reflux esophagitis, accounting 43% of clones, followed by *Firmicutes* (33%), *Bacteroidetes* (10%), *Fusobacteria* (10%), *Actinobacteria* (2%) and *TM7* (2%) (Table 2). Members of 10 genera (≧3%) were observed including *Streptococcus* (20%), *Pasteurella* (10%), *Klebsiella* (9%), *Fusobacterium* (9%), *Haemophilus* (9%), *Prevotella* (5%), *Neisseria* (4%), *Helicobacter* (3%), *Bacillus* (3%), and *Veillonella* (3%) (Figure 2B). To characterize bacterial populations in Barrett's esophagus, we analyzed bacterial flora from six patients with Barrett's esophagus. Two hundred and forty clones (40 clones from each subject) yielded 138 16S rDNA sequences, all of which were classified into 5 phyla. *Firmicutes* was the most prevalent phylum represented in Barrett's esophagus, accounting 55% of clones, followed by *Proteobacteria* (20%), *Bacteroidetes* (14%),*Fusobacteria* (9%) and *Actinobacteria* (2%) (Table 2). Members of 11 genera (≥3%) were observed, including *Veillonella* (19%), *Prevotella* (12%), *Streptococcus* (11%), *Fusobacterium* (9%), *Gemella* (4%), *Helicobacter* (4%),*Neisseria* (4%), *Actinobacillus* (4%), *Lactobacillus* (4%), *Dialister* (3%), and *Achromobacter* (3%) (Figure 2C). Compared to patients with normal esophagus or reflux esophagitis, patients with Barrett's esophagus had a lower percentage of *Streptococcus*.

Bacteria-positive patient numbers by group

To examine bacterial prevalence in the distal esophagus, we also checked positive patient numbers among the six subjects in each of the normal esophagus, reflux esophagitis, and Barrett's esophagus groups (Figure 3A, B, C). *Streptococcus*, *Provotella*, and *Helicobacter* were prevalent in all patients. Interestingly, *Veilonella*, *Neisseria*, and *Fusobacterium* were prevalent in the patients with reflux esophagitis and Barrett's esophagus, but were not found in the subjects with a normal esophagus. *Streptococcus* was the most prevalent genus in patients with a

normal esophagus (5/6), reflux esophagitis (5/6), and Barrett's esophagus (5/6). *Fusobacterium* was not detected in any subject with a normal esophagus, but was observed in five of six patients each with reflux esophagitis and Barrett's esophagus. *Helicobacter* was found in four of six subjects with a normal esophagus, two of six with reflux esophagitis, and three of six with Barrett's esophagus. These findings indicate the presence of a difference in microbial communities between normal esophagus, reflux esophagitis and Barrett's esophagus.

Discussion

Here, we analyzed bacteria colonizing the mucosal tissue in Japanese people with a normal esophagus, reflux esophagitis, and Barrett's esophagus. Our study showed no significant difference in the total amount of bacterial DNA between the three groups ($p > 0.1$), but unexpected diversity in bacterial populations on the esophageal epithelia in these subjects.

Phylum-level analysis of our present samples revealed that the bacterial communities differed among groups. First, each group had a different number of phyla: populations could by classified into four phyla in patients with normal esophagus, six in those with reflux esophagitis, and five in those

with Barrett's esophagus. Second, phyla composition differed among groups. We found differences in composition at the genus level among the normal esophagus, reflux esophagitis, and Barrett's esophagus groups. We compared not only the distribution of bacterium 16S rDNA gene clone libraries but also the bacteria-positive patient numbers. The most prevalent genus was *Streptococcus* in patients with a normal esophagus or reflux esophagitis, versus *Veillonella* in patients with Barrett's esophagus.

Conclusions

Esophageal bacterial composition differs among subjects with a normal esophagus, reflux esophagitis, and Barrett's esophagus. Diverse bacterial communities may be associated with esophageal diseases, and comparison of bacterial populations in the esophagus may enhance our understanding of the role of bacteria in esophageal diseases.