Increase in the Lipopolysaccharide Activity and Accumulation of Gram-Negative Bacteria in the Stomach With Low Acidity

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INTRODUCTION: **Lipopolysaccharides (LPSs) of Gram-negative bacteria (GNB) are highly toxic and induce inflammation. Therefore, we investigated both the LPS activity and composition of GNB in the gastric fluid (GF) to assess the potential toxicity of them accumulated in the stomach.**

METHODS: **GF and saliva samples were obtained from 158 outpatients who were undergoing upper gastrointestinal endoscopy and 36 volunteers using a nasogastric tube. The LPS activity was measured by assay kits including recombinant Factor C or *Limulus* amebocyte lysate. To assess the bacterial composition in the samples, a 16S ribosomal DNA-based operational taxonomic unit analysis was performed. We focused on the genera representing >0.1% of the whole microbiota.**

RESULTS: **We found a high LPS activity in the GF samples with weak acidity (approximately > pH 4), whereas little/no activity in those with strong acidity (approximately < pH 2). Spearman test also demonstrated a close correlation between pH and LPS in those samples ($r = 0.872$). The relative abundance of GNB in the saliva showed no significant difference between the subject groups with weak- and strong-acidity GF. In addition, in the subjects whose GF acidity was weak, the GNB abundance in the GF was almost the same as that in the saliva. By contrast, in the subjects whose GF acidity was strong, the GNB abundance in the GF was significantly lower than that in the saliva.**

DISCUSSION: **GNB that have recently moved from the oral cavity might account for the prominent LPS activity in a stomach with weak acidity.**

SUPPLEMENTARY MATERIAL accompanies this paper at https://links.lww.com/CTG/A318, links.lww.com/CTG/A319, links.lww.com/CTG/A320

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INTRODUCTION

The stomach is a harsh environment for many bacteria due to strong gastric acid. The number of culturable bacteria is at most around 10^3 colony-forming units/mL in the gastric fluid (GF) when sampled at the highest acidity in the morning after overnight fasting (1). Recently, Tsuda et al. (2) also demonstrated a significant correlation between the acidity and the number of culturable bacteria in the GF. These findings suggest that the stomach is a potential site that can be colonized by a large number of non-*Helicobacter pylori* bacteria (NHPB), but strong gastric acid inhibits the residence of those bacteria in a healthy state.

H. pylori infection is considered the trigger for the development of atrophic gastritis, which is recognized as the critical pathological step in the development of intestinal-type gastric cancer in the Correa pathway (3). Atrophic gastritis in the corpus causes the loss of parietal cells, which, thus, eventually results in a hypochlorhydric stomach and is accompanied by bacterial overgrowth there. However, whether or not such a large number of bacteria in the stomach with low acidity are linked to aggravation of atrophic gastritis and progression to gastric cancer remains unclear (4).

Lipopolysaccharides (LPSs) of Gram-negative bacteria (GNB) elicit multiple acute pathophysiological responses such as fever, endotoxin shock, and inflammation (5). The association between

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chronic inflammation and cancer, particularly those originating in the gastrointestinal (GI) system, is now established (4,6,7). Miyata et al. (8) reported that LPS extracted from NHPB, especially *Neisseria subflava*, adhering to the gastric mucosa stimulated proinflammatory cytokine secretion in a gastric epithelial cell line. They further suggested that those NHPB might perpetuate gastric mucosal inflammation and accelerate carcinogenesis, especially in a hypochlorhydric stomach. It might, therefore, be useful to measure the LPS activity in the GF to assess the potential toxicity or risk of carcinogenesis associated with the accumulation of such a bacterial mass in a stomach with weak acidity.

In this study using GF samples, we analyzed the relationship between the pH value and LPS activity in the GF. To assess the origin of LPS in the stomach, the bacterial composition in the saliva and GF was comparatively analyzed.

METHODS

Sample collection

In the first clinical study (Figure 1), 158 GF samples were obtained from outpatients who were undergoing upper GI endoscopy at the Department of Gastroenterology, Tokai University Hospital, Isehara, and Tokai University Tokyo Hospital, Tokyo, Japan. The exclusion criteria for the subjects were age younger than 20 years, suffering from acute GI or systemic diseases, and the use of antimicrobials and proton pump inhibitors (PPIs) within the previous month and week, respectively. The primary and secondary endpoints were the assays of LPS activity and the pH value in the GF, respectively. In the secondary clinical study (Figure 1), another 36 GF samples were obtained from healthy volunteers using a nasogastric tube at the Laboratory for Infectious Diseases, Tokai University School of Medicine, Isehara. The exclusion criteria were the same as those for the former study. The primary and secondary endpoints were the analysis of the bacterial composition and the measurement of pH value, respectively. The volunteers underwent both GF and saliva sampling twice at intervals of 3 months. For sampling of saliva, the stimulated saliva was collected after chewing a small piece of sterile gum for 3 minutes. All of the samples were collected from subjects in the morning after overnight fasting and immediately frozen and stored at −40 °C until the assay. The ethics committee of Tokai University School of Medicine approved the both studies (13R-324 and 18R-286), and written informed consent was obtained from all of the subjects.

Laboratory examinations including the LPS activity assay

The pH value of the GF was measured using a pH meter (M-7; Horiba, Tokyo, Japan). For the assay of the LPS activity in the GF, both the Endozyme II Recombinant Factor C Assay Kit (Hyglos GmbH, Munich, Germany) and the ToxinSensor Chromogenic *Limulus* amebocyte lysate (LAL) Endotoxin Assay Kit (GeneScript, Piscataway, NJ) were used. In the former kit, a recombinant Factor C, instead of LAL, is used in combination with a synthetic fluorogenic substrate for detection of LPS; the assay range is 0.05–50 EU/mL. The latter kit uses a modified LAL and a synthetic color-producing substrate to chromogenically detect LPS; the measurable concentration range is 0.05–1 EU/mL. In the assay, we strictly adjusted the pH value of the reaction mixture to be between 6.0 and 8.0 using endotoxin-free 0.1 N sodium hydroxide to exclude the inhibitory effect of strong acidity of GF on the reaction. The concentration of bile acids was measured using the EFBA-100 Kit (Bioassay Systems, Hayward, CA).

The 16S ribosomal DNA-based operational taxonomic unit analysis

Bacterial DNA was extracted from the GF and saliva using the Ultra Clean Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA). Using those bacterial DNA templates, the hypervariable V3–V4 region of 16S ribosomal DNA was amplified by polymerase chain reaction with 341f (9) and R806 (10) primers. Sequencing was performed using a pair-end and run on an Illumina Miseq sequencing system. The average numbers (SD) of quality filter-passed reads per 1 sample were 34,275 (4,797) and 29,374 (3,833) in 36 GF and 36 saliva samples, respectively. The high-quality reads were then sorted and grouped into operational taxonomic units (OTUs) at 97% identity using the quantitative insights into microbial ecology pipeline (11). In the bacterial composition analysis based on OTUs, we focused on the genera representing >0.1% of the total microbiota, which accounted for >85% of all of the OTUs.

Statistical analyses

The Spearman correlation coefficient (r) was adopted to evaluate the correlation between pH value and LPS activity in the GF. The Kappa statistics were measured for the agreement between those parameters in the GF. Mann-Whitney U test was used to compare the abundance of genera between the GF with strong acidity and that with weak acidity. The difference in the abundance of genera between a pair of saliva and GF samples obtained from the same subject was examined by Wilcoxon signed-rank test. All significance probability values were considered to be significant at $P < 0.05$. All statistical tests were performed using SPSS v25.0 (IBM Corp., New York).

RESULTS

Subject characteristics

The average age (SD) and men/women ratio of all the subjects for GF sampling (n = 194) included in the both clinical studies were 61.7 (14.2) years and 1.2, respectively. Endoscopic records were obtained from 140 outpatients (Figure 1), which included 50 patients with atrophic gastritis (average age, 68.5 years; men/women ratio, 1.2).

Correlation between the pH and LPS in the GF

First, we measured the LPS activity using a kit including recombinant Factor C in 136 samples, which were obtained in both the first and second studies (Figure 1). As shown in Figure 2, a high LPS activity (100–600 EU/mL) was detectable in the GF samples with a pH value more than approximately 4.0, whereas no or little activity was found in the GF with a pH of less than approximately 2.0. In the GF samples from atrophic gastritis, the average of LPS activity and pH value were 264 EU/mL and 4.7, respectively. Spearman correlation coefficient demonstrated a very close correlation ($r = 0.872$, n = 136) between the pH value and LPS activity in the samples measured by the recombinant Factor C kit. To exclude possible bias in the assay kit using recombinant Factor C, we then measured the LPS activity in another 58 samples using the prototype assay kit including LAL (Figure 1). We also found high and barely detectable levels of LPS activity in the GF with weak and strong acidity, respectively. A close correlation between the pH value and LPS activity was also found in those samples by Spearman correlation coefficient ($r = 0.749$, n = 58, data not shown).

To further confirm the significant correlation between those 2 parameters in the GF, we next examined in the second clinical study whether or not both the pH value and LPS activity changed in

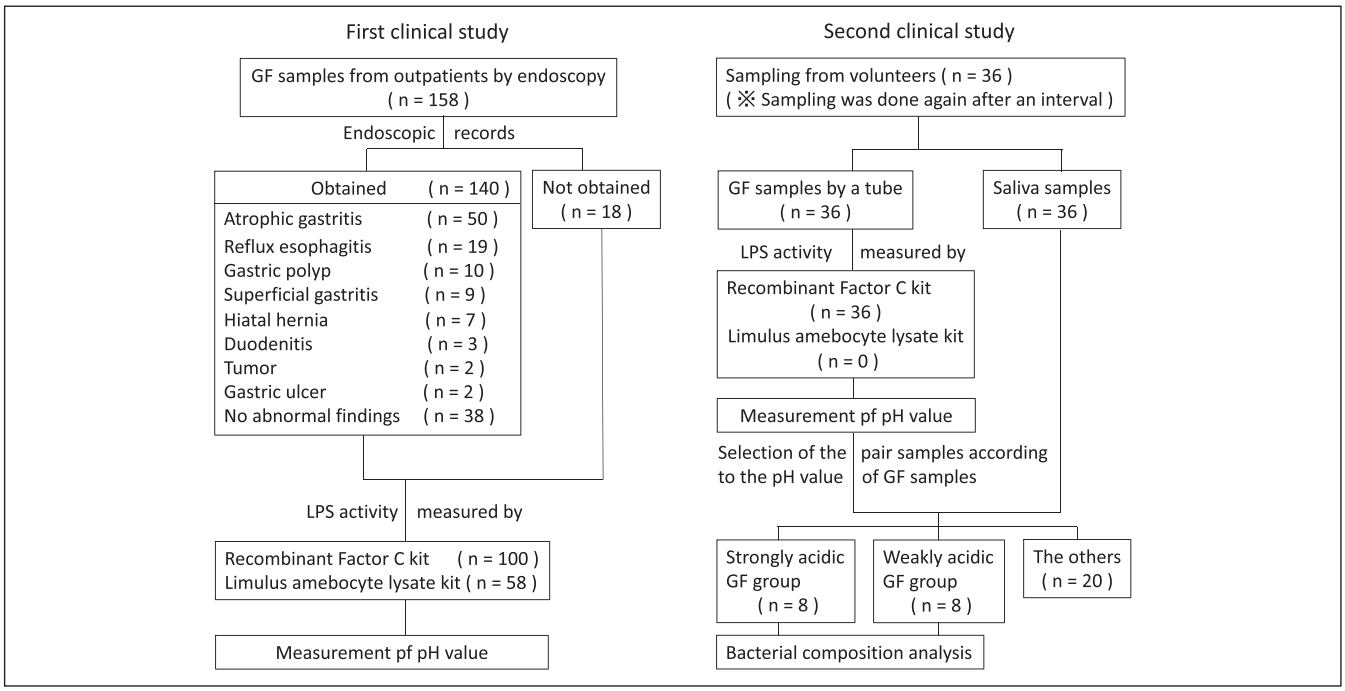

Figure 1. Flow diagram illustrating the participant flow throughout the course of the studies. GF, gastric fluid; LPS, lipopolysaccharide.

the same direction when compared between a pair of samples from the same subject. Among the 36 volunteers who underwent GF sampling twice, the LPS activity was detectable in 14 subjects before and/or after the interval, as summarized in Table 1. Agreement in the direction of change (either "increase" or "decrease" for both) between the pH value and LPS activity was observed in 13 subjects. Only 1 subject (F80) showed disagreement. The reproducibility of the agreement evaluated by the kappa coefficient was 0.891, which represented "excellent" reliability of the presumed correlation between the pH and LPS in the GF.

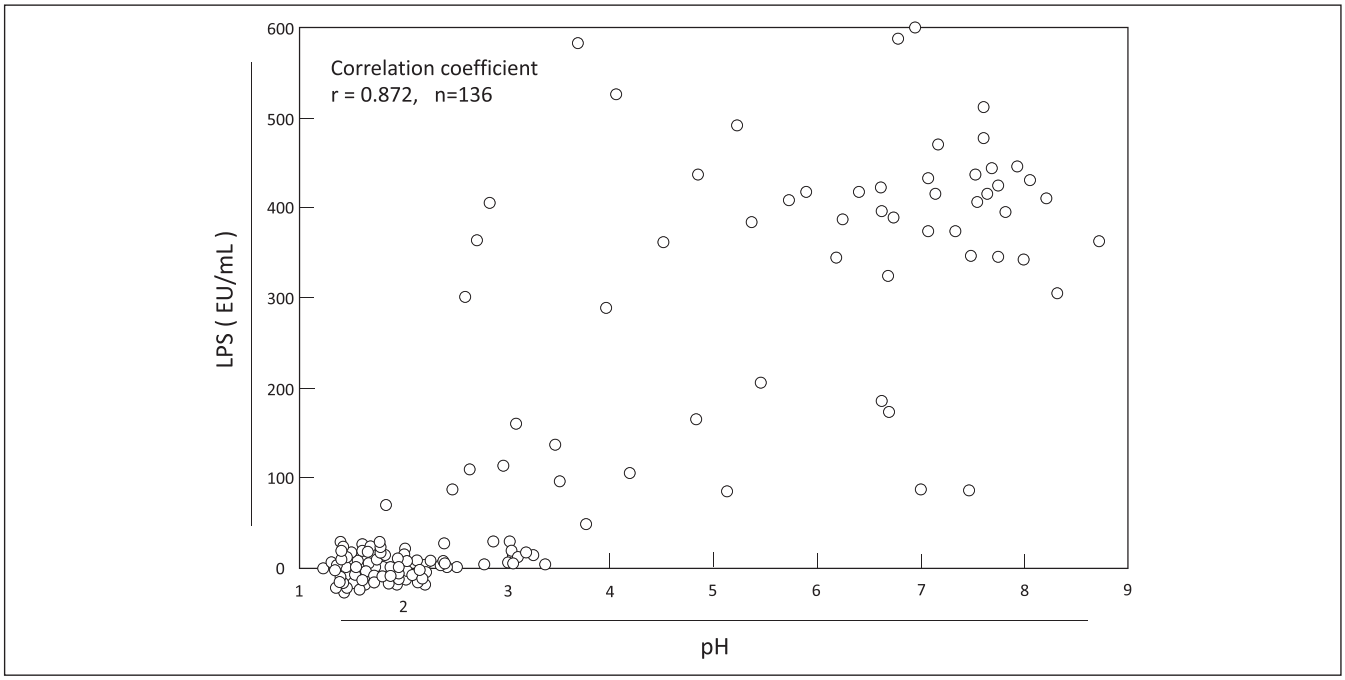

Figure 2. Correlation between the pH value and LPS activity in the GF. GF samples from 136 subjects (n) had their pH value and LPS activity measured using a recombinant Factor C assay kit. The horizontal and vertical axes indicate the pH value and LPS activity (EU/mL), respectively. Numerical values of each sample were plotted on the figure. The correlation coefficient of the both parameters by Spearman test (*r*) is shown on the upper left. GF, gastric fluid; LPS, lipopolysaccharide.

Table 1. pH value and LPS activity of the subjects before and after interval

ID	Age	Sex	pH value			LPS activity (EU/mL)		
			Before	After	Change	Before	After	Change
F63	49	F	1.68	4.22	Inc.	<0.1	21.1	Inc.
F66	47	F	4.84	2.85	Dec.	164.4	79.8	Dec.
F68	52	M	2.65	1.99	Dec.	109.0	<0.1	Dec.
F69	47	M	2.15	7.22	Inc.	<0.1	77.7	Inc.
F71	48	M	2.98	2.73	Dec.	112.4	0.4	Dec.
F72	41	F	4.20	2.34	Dec.	104.9	<0.1	Dec.
F77	55	M	3.52	1.57	Dec.	95.8	<0.1	Dec.
F79	32	M	5.14	1.81	Dec.	83.9	<0.1	Dec.
F80	47	F	7.01	8.15	Inc.	86.2	76.4	Dec.
H26	52	F	1.41	3.47	Inc.	<0.1	10.9	Inc.
H31	45	F	7.47	2.43	Dec.	85.1	<0.1	Dec.
H39	57	M	2.49	7.45	Inc.	86.1	99.2	Inc.
H41	57	F	1.70	6.71	Inc.	<0.1	88.6	Inc.
H43	57	F	2.28	1.69	Dec.	7.0	<0.1	Dec.

Dec, decreased; Inc, increased; LPS, Lipopolysaccharide.

It is likely that the LPS in GF with weak acidity might be due to reflux of the proximal small intestinal contents, including bile acids and GNB, into the stomach. However, only a weak correlation was shown between the bile acids concentration and the LPS activity in the GF by Spearman correlation coefficient ($r = 0.293$, n = 136; see Figure 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A318), whereas a moderate correlation was noted between the subject age and the LPS activity ($r = 0.352$, n = 130; see Figure 2, Supplementary Digital Content 2, http://links.lww.com/CTG/A319).

Bacterial composition in the GF and saliva

To determine the origin of GNB, which are considered to release LPS into the GF, we conducted the second clinical study, in which saliva and GF samples were obtained. Then, we constructed 2 groups according to the pH value of GF samples one group with 8 subjects (Table 2; ID, F63–H35) whose GF samples were strongly acidic (mean pH value = 1.6) and the other group with 8 subjects (F66–H39) whose GF samples were weakly acidic (mean pH = 5.0). In addition, younger subjects were assigned to both groups, in which the age was matched. Using pair samples of GF and saliva obtained from the same subject, we analyzed the bacterial composition through 16S ribosomal DNA profiling. First, we listed the 6 most-common Gram-positive and Gram-negative genera based on the relative abundance in the GF with strong acidity (Table 3). Among them, the genus *Neisseria* (Gram-negative) included not only *N. subflava* but also *N. perflava* and *N. mucosa* (data not shown). We then compared the difference in the abundance of each genus between the GF samples with strong and weak acidity. The abundance of Gram-positive bacteria (GPB) was greater in the GF with strong acidity than that in GF with weak acidity for all the 6 genera. By contrast, the abundance of GNB was greater in the GF with weak acidity than that in GF with strong acidity in 5 of the 6 genera. In line with the greater ratio of those major Gram-negative genera in the GF with weak acidity, the percentage prevalence ratio of the GNB among the genera representing >0.1% of the total microbiota was also significantly greater in the GF with weak acidity than that in GF with strong acidity, as summarized in Table 2 (% mean ratio, 65.4 vs 37.3). On the other hand, either the pH value or percentage mean ratio of GNB in the saliva was not significantly different between those 2 groups (pH value, 8.0 vs 7.8; % mean ratio, 65.6 vs 65.2, respectively).

Comparison of bacterial composition in between saliva and GF

To clarify the mechanism underlying the predominance of GNB in the GF with weak acidity, we compared the abundance of major genera in the GF with those in the saliva. Of note was that 9 of the top 10 major genera, which accounted for more than 85% of all the genera, were the same between the saliva and GF samples (Figures 3 and 4). In the subjects who had the strongly acidic GF (Figure 3), by contrast, the relative abundance was greater in the GF than that in the saliva in all of 6 Gram-positive genera (highlighted in blue color), whereas the abundance was much lower in the GF of 3 of 4 Gram-negative genera (highlighted in pink). Given that most bacteria in the GF had recently moved from the oral cavity, possibly through the inflow of the saliva, GPB and GNB from the saliva might be resistant and sensitive, respectively, in the GF with strong acidity. In the subjects who had weakly acidic GF (Figure 4), the relative abundance of both Gram-positive and Gram-negative genera was not significantly different between the GF and saliva samples, suggesting that GNB and GPB in the saliva largely survived in the GF with weak acidity.

DISCUSSION

In the analysis of GF samples obtained from 194 subjects, we detected a high LPS activity in the samples with weak acidity,

Table 2. Two groups with gastric fluid of strong and weak acidity

Subjects[a]			Saliva		Gastric fluid		
ID	Age (y)	Sex	pH	GNB (%)[b]	pH	GNB (%)	LPS (EU/mL)
F63	49	F	7.2	64.2	1.7	42.3	<0.1
F64	45	M	8.0	78.5	1.5	34.1	<0.1
F65	50	M	7.9	62.4	1.8	44.8	<0.1
F67	52	F	8.1	76.2	1.5	41.8	<0.1
F78	39	F	8.0	60.8	1.4	30.9	<0.1
H27	43	M	7.6	57.8	1.5	39.4	<0.1
H29	32	F	7.7	59.3	1.4	40.8	<0.1
H35	55	M	8.0	62.5	1.6	24.1	<0.1
45.6 ± 7.0[c]			7.8 ± 0.3	65.2 ± 7.3	1.6 ± 0.1	37.3 ± 6.6	<0.1
F66	47	F	8.0	67.1	4.8	50.1	166.4
F71	48	M	7.9	73.5	4.0	87.4	112.4
F72	41	F	7.8	56.0	4.2	61.3	104.9
F77	55	M	7.9	62.6	3.5	72.0	95.8
F79	32	M	7.8	64.6	5.1	58.3	83.9
F80	47	F	7.9	71.1	7.0	69.4	86.2
H31	45	F	8.0	71.9	7.5	67.8	85.1
H39	57	M	8.4	57.9	3.5	56.8	86.1
46.5 ± 7.3			8.0 ±0.2	65.6 ± 6.1	5.0 ± 1.4[d]	65.4 ± 10.8[d]	102.6 ± 26.0[d]

GNB, Gram-negative bacteria; LPS, lipopolysaccharide.
[a]Selected from 36 volunteers.
[b]% prevalence ratio.
[c]Mean ± SD.
[d]Significant intergroup difference.

whereas little activity was detected in those with strong acidity. Spearman test also demonstrated a very close correlation between the pH value and LPS activity in those GF samples.

The LPS-induced clotting phenomenon of LAL is so specific and sensitive that it has been used to detect even minimal amounts of LPS. The cardinal receptor for this coagulation cascade is the protein

Table 3. Comparison in the relative abundance of bacteria at the genus level

		Relative abundance (%) in the gastric fluid with			
Genus	Gram	Strong acidity	Comparison	Weak acidity	Difference
Streptococcus	P	32.9 (31.8–38.7)[a]	>	23.4 (17.2–27.6)	S
Rothia	P	4.8 (3.4–8.7)	>	2.4 (1.4–2.7)	S
Actinomyces	P	4.2 (3.5–4.4)	>	2.9 (2.0–3.4)	S
Granulicatella	P	3.0 (2.4–3.7)	>	2.4 (1.4–3.2)	NS
Lactobacillus	P	2.9 (1.4–9.7)	>	0.1 (0.0–0.2)	S
Gemella	P	1.4 (1.1–1.6)	>	1.3 (1.0–1.8)	NS
Veillonella	N	14.0 (11.8–15.9)	>	10.0 (6.9–13.6)	NS
Prevotella	N	7.7 (7.3–8.8)	<	19.6 (15.8–24.4)	S
Neisseria	N	7.3 (5.4–8.2)	<	9.4 (7.3–12.7)	NS
Haemophilus	N	6.5 (5.6–6.8)	<	9.5 (6.0–11.6)	NS
Fusobacterium	N	0.7 (0.5–0.9)	<	4.4 (2.8–6.6)	S
Porphyromonas	N	0.06 (0.0–0.1)	<	1.1 (0.4–2.4)	S

N, negative; NS, no significant difference; P, Positive; S, significant difference ($P < 0.05$).
[a]Mean (interquartile range), n = 8.

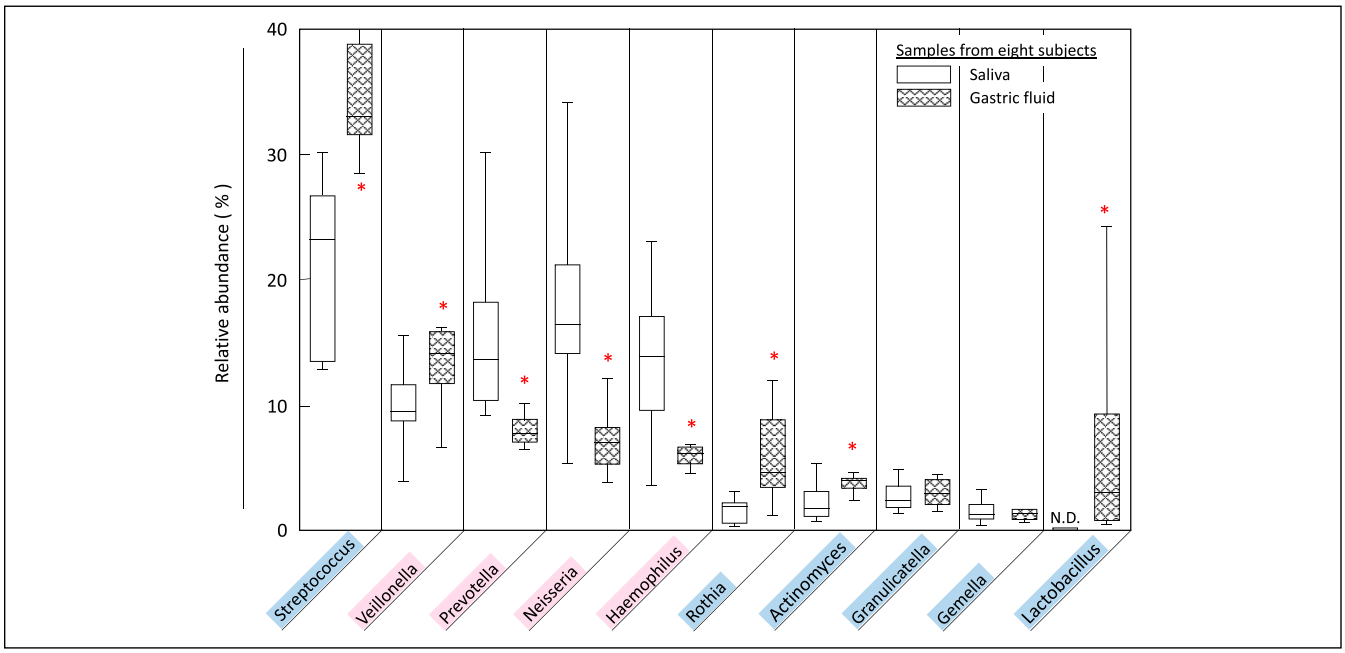

Figure 3. Comparison of the bacterial composition in the saliva with that in the GF with strong acidity. A pair of saliva and GF samples was obtained from 8 subjects, whose GF samples showed strong acidity (Table 2; ID, F63–H35), and the bacterial composition of these samples was analyzed through 16S ribosomal DNA profiling. The 10 most-common genera in the GF and saliva are shown on the figure by a box-and-whisker plot. The names of the genera are shown at the bottom, and the Gram-positive and Gram-negative ones are highlighted in blue and pink, respectively. A red asterisk indicates a significant difference in the relative abundance between saliva and GF in each genus. GF, gastric fluid; N.D., not detected.

named Factor C, a proenzyme that is included in LAL and, thus, activated by LPS (12). In this study, we used assay kits using recombinant Factor C and LAL to measure the LPS activity in the GF. Among parameters that potentially influence the test for LPS activity, the pH and proteases such as pepsin in the GF samples had to be considered. To exclude the influence of a low pH in the GF, we adjusted the pH of the reaction mixture to the neutral range. The nonspecific protease activity was evaluated by confirming that the reaction curve in the assay was Factor C specific. With those adjustments, we confirmed a high LPS activity in the GF with weak acidity.

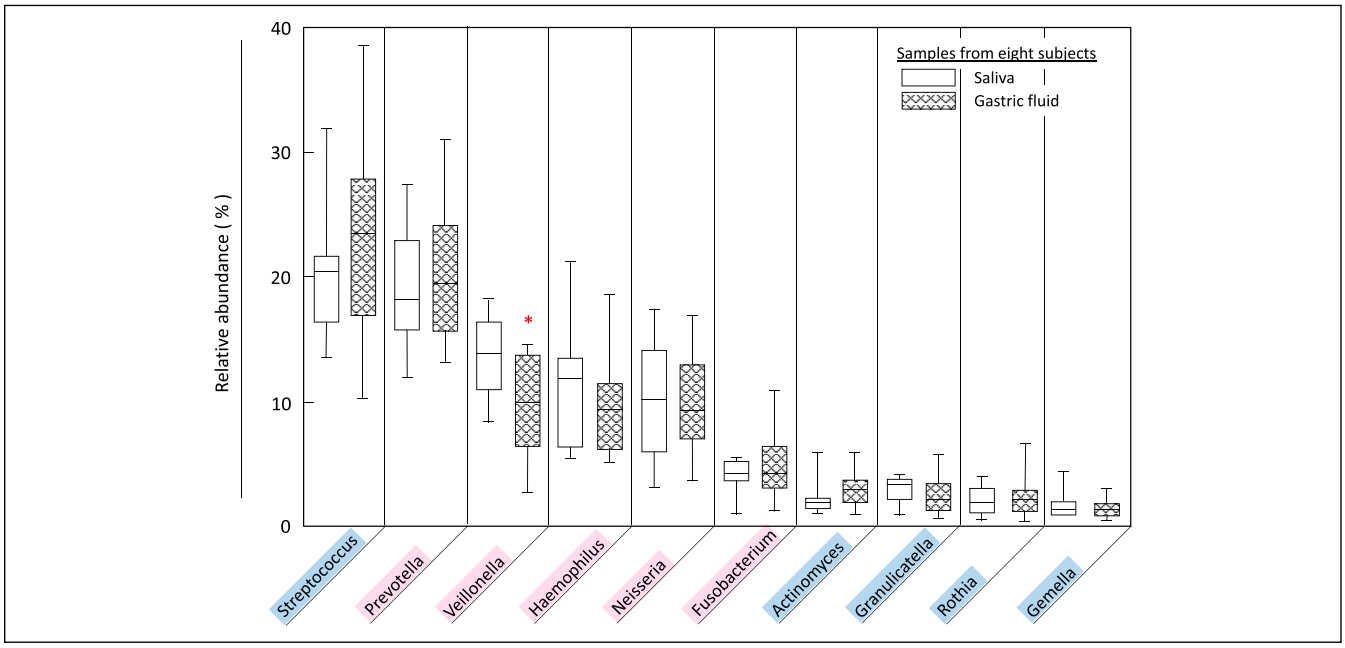

Figure 4. Comparison of the bacterial composition in the saliva with that in the GF with weak acidity. A pair of saliva and GF samples was obtained from 8 subjects whose GF samples showed weak acidity (Table 2; ID, F66-H39) and processed in the same way as described in the legend of Figure 3.

Helicobacter pylori infection is usually acquired early in life and is followed by a long phase of chronic inflammation of gastric mucosa. Peptic ulcer tends to develop in *H. pylori*-infected patients at an earlier age than in those suffering from gastric cancer (13). Of note is that the patients who develop *H. pylori*-associated duodenal ulcers seem to be protected from developing cancers (14,15). The mechanism underlying such protection involves a higher level of gastric acid secretion (4). Therefore, hypochlorhydria, which eventually follows the development of atrophic gastritis, could be a major risk factor for the occurrence of gastric cancers. Moreover, such a stomach with weak/no acidity is a site for bacterial overgrowth, which is suspected to perpetuate gastric inflammation and accelerate neoplastic progression (16). In this study, we clearly showed that potentially carcinogenic LPSs were accumulated in high amounts in the GF of the hypochlorhydric stomach. Because Toll-like receptor 4 is sensitized by LPS and upregulated in cancer tissue, its signaling further activates proinflammatory cascades and might be involved in the development of gastric cancer (17).

Although *H. pylori* eradication has been shown to reduce the risk of gastric cancer occurrence, a considerable number of these individuals still continue to develop gastric cancer even after the successful eradication (18). According to an epidemiological study using a health database of Hong Kong, long-term use of PPI was still associated with an increased gastric cancer risk in subjects even after *H. pylori* eradication therapy (19). It is likely that the direct suppression of acid-producing parietal cells by PPI worsens atrophic gastritis, which then leads to acceleration of the carcinogenesis in the stomach (20). However, it is also possible that long-term PPI use induces a weakly acidic state and resultant high LPS activity in the stomach, which might aggravate gastric inflammation, resulting in the development of gastric atrophy and cancers.

In the analysis of the bacterial composition in the GF with weak acidity, the relative abundance of GNB, which were the source of LPS, was about twice that in the GF with strong acidity. GNB were generally more sensitive to acids than GPB. The times necessary to inactivate 90% of the initial burden in 5% hydrochloric acid were 3.7 min and 10.9 min in *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive) strains, respectively (21), suggesting that most of the GNB survived killing in the stomach with weak acidity. In addition, because the difference in the pH value between the higher and lower pH groups was more than 3.0 (Table 2), the number of live (and culturable) GNB was estimated to be approximately 1,000 times greater in the former than in the latter groups, according to the report by Tsuda et al. (2) Taken together, the actual number of live GNB was supposed to be approximately 2,000 times greater in the higher pH than that in the lower pH groups, which will account for the prominent LPS activity in the GF with weak acidity.

In the study exploring mucosal microbiome dysbiosis in gastric carcinogenesis, Coker et al. (22) found a significant enrichment of GNB such as *Prevotella*, *Peptostreptococcus*, and *Fusobacterium* in patients with gastric cancer. Because those GNB are known to be commensals inhabiting the oral cavity, the authors addressed the need for a comparative analysis of microbiomes in the gastric and salivary samples to delineate the role of those bacteria in the tumorigenesis. In addition, in our comparative analysis of the bacteria in the GF with those in the saliva, the GNB in the GF were strongly suggested to have come from the oral cavity through the continuous inflow of the saliva (2).

Fluctuation of the pH value and LPS activity in the same subjects at an interval (Table 1) suggested the possibility of intervention to remove LPS. Given that the accumulation of LPS in the stomach is induced by its low acidity, increasing the acidity by probiotics might help stop the increase in LPS activity. Now, a lot of lactobacillus strains secreting lactic acid are widely used as probiotics. The probiotics targeting the stomach are required to remain the stomach for some time without being inactivated by gastric acid (23). Finally, the limitations of this study include the absence of *H. pylori* status in the subjects and the shortness of the detail in the difference of clinical characteristics between strongly and weakly acidic groups.

CONFLICTS OF INTEREST

Guarantor of the article: Yasuhiro Koga, MD.
Specific author contributions: M.S., T.U., M.I., J.K., M.F., H.M., M. Monma, E.T., S.Y., H.S., M. Morimachi, A.I., T.U., and K.S. recruited the patients, performed endoscopy, and sampled gastric fluid. T.M. performed biochemical analyses. M.K. performed statistical analysis. M. Matsushima and T.S. were involved in the study concept and design. Y.K. was involved in the study concept and design, data interpretation, and drafting the manuscript.
Financial support: Meiji Co., Ltd Research Aid.
Potential competing interests: None to report.

Study Highlights

WHAT IS KNOWN

- ✓ LPSs of GNB induce inflammation and promote carcinogenesis.
- ✓ Atrophic gastritis caused by *H. pylori* infection eventually results in hypochlorhydria accompanied by bacterial overgrowth in the stomach.

WHAT IS NEW HERE

- ✓ A high LPS activity was found in the GF samples with weak acidity.
- ✓ In the subject with weak GF activity, the GNB abundance in the GF was almost the same as that in the saliva.
- ✓ These results suggested that GNB from the oral cavity might account for the prominent LPS activity in the stomach with weak acidity.

TRANSLATIONAL IMPACT

- ✓ Increasing the acidity might help reduce the LPS activity and, thus, prevent carcinogenesis in the hypochlorhydric stomach.

REFERENCES

1. Martinsen TC, Bergh K, Waldum H. Gastric juice: A barrier against infectious diseases. Basic Clin Pharmacol Toxicol 2005;96:94–102.
2. 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.
3. Correa P. Helicobacter pylori and gastric carcinogenesis. Am J Surg Pathol 1995;19(Suppl 1):S37–43.
4. Fox JG, Wang TC. Inflammation, atrophy, and gastric cancer. J Clin Invest 2007;117:60–9.
5. Rietschel ET, Brade L, Braudenburg K, et al. Chemical structure and biological activity of bacterial and synthetic lipid A. Rev Infect Dis 1987; 9(Suppl 5):527–36.

6. McMillan DC, Canna K, MaArdle CS. The effect of deprivation and the systemic inflammatory response on outcome following curative resection for colorectal cancer. Br J Cancer 2003;89:612–4.
7. Terzic J, Grivennikov S, Karin E, et al. Inflammation and colon cancer. Gastroenterology 2010;138:2101–14.e5.
8. Miyata N, Hayashi Y, Hayashi S, et al. Lipopolysaccharides from non-Helicobacter pylori gastric bacteria potently stimulate interleukin-8 production in gastric epithelial cells. Clin Transl Gastroenterol 2019;10: e00024.
9. Muyzer G, de Waal EC, Uitterlinden AG. Profiling of complex mirobial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S eRNA. Appl Environ Microbiol 1993;59:695–700.
10. Caporaso JG, Lauber CL, Walters WA, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 2011;108(Suppl 1):4516–22.
11. Igarashi M, Nakae H, Matsuoka T, et al. Alteration in the gastric microbiota and its restoration by probiotics in patients with functional dyspepsia. BMJ Open Gastro 2017;3:e000144.
12. Nakamura T, Morita T, Iwanaga S. Lipopolysaccharide-sensitive serine-protease zymogen (factor C) found in Limulus hemocytes. Eur J Biochem 1986;154:511–21.
13. Suerbaum S, Michetti P. Helicobacter pylori infection. N Engl J Med 2002; 347:1175–86.
14. Hansson LE, Nyren O, Hsing AW, et al. The risk of stomach cancer in patients with gastric or duodenal ulcer disease. N Engl J Med 1996;335:242–9.
15. Uemura N, Okamoto S, Yamamoto S, et al. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med 2001;345:784–9.
16. Stockbruegger RW. Bacterial overgrowth as a consequence of reduced gastric acidity. Scand J Gastroenterol 1985;111:7–16.
17. Oblak A, Jerala R. Toll-like receptor 4 activation in cancer progression and therapy. Clin Dev Immunol 2011;2011:609579.
18. Lee YC, Chiang TH, Chou CK, et al. Association between Helicobacter pylori eradication and gastric cancer incidence: A systemic review and meta-analysis. Gastroenterology 2016;150:1113–24.
19. Cheung KS, Chan EW, Wong AYS, et al. Long-term proton pump inhibitors and risk of gastric cancer development after treatment for Helicobacter pylori: A population-based study. Gut 2018;67:28–35.
20. Kuipers EJ, Lundell L, Klinkenberg-Knol EC, et al. Atrophic gastritis and Helicobacter pylori infection in patients with reflux esophagitis treated with omeprazole or fundoplication. N Engl J Med 1996;334:1018–22.
21. Mazzola PG, Martins AMS, Penna TCV. Chemical resistance of the gram-negative bacteria to different sanitizers in a water purification system. BMC Infect Dis 2006;6:131.
22. Coker OO, Dai Z, Nie Y, et al. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut 2018;67:1024–32.
23. Koga Y, Ohtsu T, Kimura K, et al. Probiotic L. gasseri strain (LG21) for the upper gastrointestinal tract acting through improvement of indigenous microbiota. BMJ Open Gastro 2019;6:e000314.