CASE REPORT

Unidentified intralesional and intracellular coccoid microorganism discovered in the young man with a diffuse erosive gastroduodenitis and multiple superficial ulcerations

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A *Helicobacter pylori*-negative young Japanese man with dyspeptic symptoms suffered from a diffuse erosive gastroduodenitis and multiple superficial ulcerations. Histology and electron microscopic examinations on the biopsy specimens revealed the presence of multiple unidentified intralesional and intracellular coccoid microorganisms in the pathological gastroduodenal mucosa. Microaerophilic and anaerobic Gram-negative coccoid and filamentobacillary bacteria were cultured from the gastric aspirate. The triple therapy containing tetracycline for 14 days followed by 4 months treatment with omeprazole resulted in the resolution of the gastroduodenal pathology. The question, therefore, was raised regarding a possible role for the cultured coccoid bacteria in the pathogenesis. 16S rRNA gene sequence analysis of the isolated Gram-negative coccoid bacteria revealed a close relationship with *Haemophilus haemolyticus*. The unidentified coccoid microorganisms and cultured X and V factors independent coccoid bacteria, however, shared similar phenotypic, microbiological and pathological characteristics to the novel Gram-negative *Streptococcaceae*: *Okadaella gastrococcus*.

BACKGROUND

Various bacterial, viral, protozoan and fungal microorganisms can be detected in the human stomach. For instance, the members of the bacterial genera are *Actinomyces*, *Aspergillus*, *Bacteroides*, *Candida*, *Corynebacterium*, *Cryptosporidium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Fusobacterium*, *Gemella*, *Giardia*, *Haemophilus*, *Helicobacter*, *Histoplasma*, *Klebsiella*, *Lactobacillus*, *Mycobacterium*, *Neisseria*, *Ochrobactrum*, *Okadaella*, *Peptostreptococcus*, *Prevotella*, *Rothia*, *Staphylococcus*, *Streptococcus*, *Treponema* and *Veillonella*.^{1–7}

Among these bacteria, *Helicobacter pylori*, *Helicobacter heilmannii* and *Helicobacter felis* are human gastric pathogens.¹ In particular, *H pylori* has been investigated extensively because of its association with various human pathology, including gastroduodenitis, peptic ulcer disease and gastric malignancies.⁸ Morphologically, there are two types of *H pylori*: one is the motile and culturable spiral S-shaped bacillary form and the second is the non-motile and unculturable coccoid form.⁹ Furthermore, there are international disagreements with coccoid forms of *H pylori* as to whether they are viable

dormant forms¹⁰ or non-viable degenerative forms¹¹ in spite of the association with the development of gastric adenocarcinoma.¹² Considering the increase in *H pylori*-negative gastropathy with unknown aetiology,^{13 14} the lack of viable intralesional and intracellular coccoid *H pylori*⁹ questions us whether any other coccoid microorganisms might be involved in the process.

We report a case of the *H pylori*-negative young Japanese man with a diffuse erosive gastroduodenitis and multiple superficial ulcerations associated with unidentified intralesional and intracellular coccoid microorganisms. Possible candidate of the coccoid microorganism is discussed in this report as well as clinical, endoscopic, histological, ultrastructural, microbiological and molecular findings are described.

CASE PRESENTATION

We present the case of a 20-year-old Japanese man reported with dyspeptic symptoms, indigestion, heartburn and an occasional history of gastro-oesophageal reflux in late September 1996. He denied abdominal bloating, nausea, vomiting and melaena. There were episodes of diarrhoea 3–4 times/day when he was found to suffer from a diffuse erosive gastroduodenitis and multiple superficial ulcerations. He did not use non-steroidal anti-inflammatory drugs, smoked 3–10 cigarettes daily and drunk 375 mL beer daily. There was no history of peptic ulcer disease or gastrointestinal malignancy in his family.

INVESTIGATIONS

All routine blood tests including full blood counts, biochemical parameters and inflammatory markers were within normal limits. As his symptoms did not resolve with famotidine 40 mg/day over 6 weeks, he had an endoscopic examination and biopsy in early November. The endoscopic findings were haemorrhagic diffuse erosive gastroduodenitis and multiple superficial ulcerations (figure 1A,B). The histology showed a florid active chronic gastritis with focal surface erosion (figures 1C and 2A). Unidentified coccoid microorganisms were visualised as numerous round “dots” occurring singly, in pairs and in short chains, in gastric erosion (figure 2B), gastric pits (figure 2C,D) and gastric epithelial cells (figure 2D) in H&E and Warthin-Starry silver (WSS)-stained sections. Filamentobacillary microorganisms were seen

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Figure 1 The endoscopic views and H&E-stained gastric biopsy specimen. The endoscopy views showing multiple gastric erosions (A) and a large duodenal ulcer with partial ischaemic and necrotic area (B). The histology of the gastric biopsy showing a florid active chronic gastritis (C, magnification $\times 187.5$).

only in the mucous layer with WSS stain. A cluster of unidentified coccoid microorganism was found in the erosive lesions of the gastric mucosa (figure 3A) by scanning electron microscope. The intralesional and intracellular coccoid microorganisms with fimbriae and unipolar flagella (figure 3B) were found in the damaged gastric mucosa (figure 3B) and goblet cells in the inflamed duodenum (figure 3C) by transmission electron microscope (TEM). In the mean time, histology, immunohistochemistry and electron microscopy were negative for *H pylori*.

In February 1997, though cultures were all negative for *H pylori*, we cultured Gram-negative coccoid and Gram-negative filamentobacillary bacteria from the gastric aspirate when he still suffered from chronic gastritis with microscopic focal erosion. Identification of the bacteria was not possible by commercially available identification kits. The

bacteria were urease, catalase and oxidase negative and arginine aminopeptidase positive. Urease tests on biopsy specimens in Christensen's urea broths over 2 weeks were all negative. The colonies were not fluorescent under ultraviolet light. They were sensitive to tetracycline, erythromycin and penicillin, and resistant to metronidazole.

The partial 16S rRNA gene sequence analysis performed by Dr L Sly, the Department of Microbiology at the University of Queensland, showed that neither the coccoid bacteria nor filamentobacillary bacteria were phylogenetically related to *H pylori*, but closely related to *Haemophilus haemolyticus* (99.2%) and *Haemophilus parainfluenzae* (99.4%), respectively. However, tests for the factors X and V, which are growth requirements for *H haemolyticus* and *H parainfluenzae*,¹⁵ were negative.

TREATMENT

The triple therapy consisting of tetracycline 500 mg thrice daily, metronidazole 200 mg thrice daily and famotidine 40 mg twice daily was provided for 2 weeks, and famotidine 40 mg daily was continued until the end of December. Famotidine was substituted with omeprazole 20 mg twice daily from January 1997.

OUTCOME AND FOLLOW-UP

Following the treatment with omeprazole 20 mg twice daily for 4 months, the unremarkable and restored gastroduodenal morphology was identified at endoscopic examination in May 1997. At present, he remains asymptomatic and is fit and well.

DISCUSSION

Symptomatic and microscopic improvement of this patient occurred after 4 months of treatment with omeprazole following the triple therapy for 2 weeks. The patient has not had a recurrence of his condition and remains symptom-free for over 16 years (personal communication). Therefore, the combination therapy could have resulted in the resolution of his conditions. The contribution of omeprazole in his recovery could be explained by creation of unfavourable environment to suppress the growth of unidentified coccoid microorganisms as it had been seen for *H pylori*.^{16 17} McGowan *et al*¹⁸ also described a non-selective and urease-independent antibacterial activity of omeprazole. Subsequently, the suppressant and inhibitory mechanisms of omeprazole on unidentified coccoid microorganisms might have contributed to the improvement of his conditions by a reduction of the microorganisms and inhibition of acid effects over a 4-month period following the triple therapy and providing an optimal environment for the host's natural defence system to respond and resolve the damaged gastric mucosa.

The unidentified intralesional and intracellular coccoid microorganisms are unique and the novel bacteria had been previously unknown. The main reason as to why these microorganisms had not been discovered earlier was due to difficulty in visualising the bacteria on H&E-stained histological preparation with standard microscopy. They stain similar to the background colour and are small in size (0.3–0.5 μm in diameter). The H&E staining method predominantly is used for diagnosing purpose. Most histopathologists do not routinely use a magnification greater than $\times 400$, unless a suspected pathogen is identified. Under lower magnification, the bacteria appeared like cellular debris of cytoplasmic contents of gastric mucosal cells such as chromatin granules, lysosomes, phagosomes and mucosal vesicles.

Figure 2 Histological examination of gastric biopsy specimens. H&E-stained section in (A) (magnification $\times 600$) showing the site of gastric erosion. Multiple intralesional coccoid microorganisms that are indicated by arrows in (B) (magnification $\times 1500$) are just visible in the area marked by an asterisk in (A) under Normaski differential interference contrast microscopy. Multiple coccoid microorganisms are seen in the gastric pits of H&E-stained section in (C) (magnification $\times 1875$) and Warthin-Starry silver (WSS) stained sections in (D) (magnification $\times 1500$) in the areas marked with asterisks. Intracellular coccoid microorganisms, which are marked by arrows, are seen in WSS-stained section in (D).

Various coccoid microorganisms, previously, may have been seen under the standard microscopy and regarded as nasooropharyngeal contaminants or commensals. Nevertheless, the unidentified coccoid microorganisms found by electron microscopy are very unlikely just to be nasooropharyngeal contaminants as they could colonise the human stomach beneath the mucous layer, on the surface of gastric epithelial cells, in gastric pits, in gastric erosive lesions and superficial ulcers. It is possible

that a small number of these microorganisms may not cause histologically noticeable gastric mucosal cell damage and may colonise the stomach as if they were intracellular commensals.

Electron microscopic studies confirmed the colonisation of coccoid forms of *H pylori* in the human stomach.^{19 20} The novel bacteria, consequently, might have been mistaken as the coccid *H pylori*. Noach *et al*²⁰ reported that 17% of antral biopsy specimens and 35% of duodenal specimens contained

Figure 3 Ultrastructural studies of the biopsy specimens. The unidentified coccoid microorganisms are seen in the microerosion of the gastric mucosa (A). The phagocytotic activity of a leucocyte (L) is seen at the site of the erosion by scanning electron microscope. Multiple coccoid microorganisms are seen not only on the surface of the erosion and the inside of the damaged gastric mucosa (B), but also on the goblet cell (C, original magnification $\times 3000$) of duodenitis by transmission electron microscope. The coccoid microorganisms possess fimbriae and flagella (F). The arrows indicate the coccoid microorganisms. White bars equal 1 μm .

coccoid forms of *H pylori* in their study. Therefore, an accurate diagnosis of the coccoid microorganisms that are associated with the gastroduodenal pathology is essential, as the choice of treatment and management of the patient would be different.

The survival mechanism of the unidentified coccoid microorganisms in the gastric acidic environment is not clear. It is speculated that a clumped mass of the coccoid microorganisms with unipolar flagella may move swiftly underneath the gastric mucous layer to avoid gastric acid exposure and then into the gastric mucosa. The pathogenesis of erosive gastroduodenitis and superficial ulcerations associated with colonisation of the unidentified coccoid microorganisms is not known. It may be suggested by their morphological structure because they appeared to be adherent to the epithelial surface utilising fimbriae, which might cause damage to the gastric and duodenal epithelia. This adherence is a criterion for the pathogenicity of certain bacteria.^{19 21} For example, the fimbriae-mediated adherence of bacteria to the epithelial cell surface is an important initial step in the initiation of pathogenesis of bladder ulcer and cystitis.²¹ The additional pathogenesis must be considered because TEM showed that the presence of unidentified intralesional and intracellular coccoid microorganisms was associated with the cytoplasmic destruction and an erosive detachment of the gastric mucosa. Furthermore, the ultrastructural studies showed that the presence of the microorganisms was not only intralesional and intracellular but also present on the luminal side of the apical cell surface. The coccoid microorganisms intimately adhered to, or were juxtaposed to, the surface mucosal cells. Ogata and Araki²² observed that *H pylori* was preferentially located on the luminal side of the apical junction complexes associated with a 'dome-like bulging' of the apical cytoplasm without interruption of epithelial continuity. Therefore, a different pathogenic mechanism at the cellular level of the damaged surface mucosal cells seems to be involved in the case of the invasive unidentified coccoid microorganisms.

Considering the results of partial 16S rRNA gene sequence study and phylogenetic analysis, the coccoid and filamentobacillary bacteria were closely related to *Haemophilus haemolyticus* and *Haemophilus parainfluenzae*, respectively. They are commonly found in the nasopharyngeal cavity. Hence, there is a chance that cultured and isolated microorganisms also could be contaminants from the naso-oro-pharyngeal cavity, although he had a demonstrable pathology. However, isolated bacteria grow without X and V factors, which are essential for *H haemolyticus* and *H parainfluenzae* in their growth. Furthermore, *Haemophilus* species do not possess a flagellum, which was seen on isolated bacteria under electron microscopy. Therefore, DNA-DNA hybridisation analyses with the most closely related *Haemophilus* species with isolated coccoid and filamentobacillary bacteria are necessary. The analyses also help to confirm whether the unidentified intralesional and intracellular invasive microorganisms found in the present gastroduodenal pathology are identical to the cultured bacteria. Thus, the possibility remains that the unidentified intralesional and intracellular coccoid microorganisms are different from the cultured bacteria.

Judging from phenotypic features, including Gram-negativity, size, growth characteristics, biochemical test results, antibiotic sensitivity and morphology, including unipolar flagella and fimbriae in addition to the pathology found in this patient by histology and ultrastructural investigations, cultured bacteria do not fit into the known genus *Haemophilus*. These microorganisms, on the other hand, very closely resemble a newly identified Gram-negative *Streptococcaceae*: *Okadaella gastrococcus* (ATCC BAA-2258, NBRC 107862, V10/022878, GenBank HQ699465).⁷ It is very interesting to note that intralesional and

intracellular *O gastrococcus* has been found not only in erosive lesions of the gastroduodenal mucosa among *H pylori*-negative²³ and *H pylori*-positive²⁴ young generations, but also in the classic gastric carcinogenic cascade.^{25 26} The pathogenesis of the haemorrhagic diffuse erosive gastroduodenitis and multiple superficial ulcerations associated with unidentified coccoid microorganisms may be explained by that of intralesional and intracellular *O gastrococcus*-associated gastric mucosal lesions.^{27 28}

The transmission route and the reservoir for this microorganism are not certain. Lee²⁹ suggested that cat, dog, ferret, primates and cheetah were reservoirs for *Helicobacter* species. He did not keep or have close encounter with animals. Therefore, animals are unlikely to be reservoirs for the novel bacteria.

In conclusion, the unidentified intralesional and intracellular coccoid microorganisms and cultured bacteria discovered in this case of a diffuse erosive gastroduodenitis and multiple superficial ulcerations were not the coccoid form *H pylori*, but share similar phenotypic, microbiological and pathological characteristics with *O gastrococcus*. The new coccoid bacteria which were cultured and isolated from the patient are suspected as being responsible for the pathology. However, all Koch's postulates must be satisfied for this to be the pathogen of a diffuse erosive gastroduodenitis and multiple superficial ulcerations.

Learning points

- ▶ Various microorganisms, including bacteria, viruses, protozoa and fungi can be detected in the human stomach.
- ▶ There is an increase in *Helicobacter pylori*-negative gastropathy with unknown aetiology, although *H pylori* is the most common pathogenic gastric bacteria at present.
- ▶ Though electron microscopy on gastric biopsy specimens is not a routine investigation, it is a very useful tool to identify a possible pathogen when one suspects the involvement of unknown microorganism.
- ▶ Consider the diagnosis of a diffuse erosive gastroduodenitis with multiple superficial ulcerations in *H pylori*-negative patients who present with dyspeptic symptoms, indigestion, heartburn, a history of gastroesophageal reflux and the recent onset of diarrhoea.
- ▶ The triple therapy consisting of tetracycline, metronidazole and famotidine followed by omeprazole more than 4 months was an effective therapy for this *H pylori*-negative young patient with a diffuse erosive gastroduodenitis and multiple superficial ulcers.

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REFERENCES

- O'Rourke J, Lee A, Kellow JE. *Helicobacter heilmannii* and other gastric infections of humans. In: Blaser MJ, Smith PD, Ravdin JJ, et al, eds. *Infections of the gastrointestinal tract*. 2nd edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2002:523–33.
- Bik EM, Eckburg PB, Gill SR, et al. Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci USA* 2006;103:732–7.
- Hosaka Y, Okamoto R, Irinoda K, et al. *Helicobacter pylori* may survive ampicillin treatment in the remnant stomach. *J Antibiot (Tokyo)* 2002;55:495–8.
- Sterling CR, Guerrant RL. *Cryptosporidium*. In: Blaser MJ, Smith PD, Ravdin JJ, Greenberg HB, Guerrant RL, eds. *Infections of the gastrointestinal tract*, 2nd edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2002:1007–27.
- Dharne MS, Misra SP, Misra V, et al. Isolation of urease-positive *Ochrobactrum intermedium* in the stomach of a non-ulcer dyspeptic patient from north India. *J Microbiol Immunol Infect* 2008;41:183–6.
- Lee SG, Kim C, Ha YC. Successful cultivation of a potentially pathogenic coccoid organism with trophism for gastric mucin. *Infect Immun* 1997;65:49–54.
- Okada T, Adkins G, Hori K, et al. *Okadaella gastrococcus*, Gram-negative *Streptococcaceae*: evidence should be classified as a new microorganism. In: ICAAC2013 abstracts: 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, 10–13 September 2013, Denver, CO: the American Society for Microbiology and Omnipress; 2013:215(D-1661).
- Asaka M, Sepulveda AR, Sugiyama T, et al. Gastric cancer. In: Mobley HLT, Mendz GL, Hazell SL, eds. *Helicobacter pylori physiology and genetics*. Washington, DC: ASM Press, 2001, 481–98.
- Testerman TL, McGee DJ, Mobley HLT. Adherence and colonization. In: Mobley HLT, Mendz GL, Hazell SL, eds. *Helicobacter pylori physiology and genetics*. Washington, DC: ASM Press, 2001:381–417.
- Cellini L, Allocati N, Angelucci D, et al. Coccoid *Helicobacter pylori* not culturable *in vitro* reverts in mice. *Microbiol Immunol* 1994;38:843–50.
- Kusters JG, Gerrits MM, Van Strijp JA, et al. Coccoid forms of *Helicobacter pylori* are the morphologic manifestation of cell death. *Infect Immun* 1997;65:3672–9.
- Chan WY, Hui PK, Leung KM, et al. Coccoid forms of *Helicobacter pylori* in the human stomach. *Am J Clin Pathol* 1994;102:503–7.
- Xia HH, Wong BC, Wong KW, et al. Clinical and endoscopic characteristics of non-*Helicobacter pylori*, non-NSAID duodenal ulcers: a long-term prospective study. *Aliment Pharmacol Ther* 2001;15:1875–82.
- Yuan Y, Padol IT, Hunt RH. Peptic ulcer disease today. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:80–9.
- Kilian M. *Haemophilus*. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, eds. *Manual of clinical microbiology*, 9th edn. Washington, DC: ASM Press, 2007:636–48.
- Hui WM, Lam SK, Ho J, et al. Effect of omeprazole on duodenal ulcer-associated antral gastritis and *Helicobacter pylori*. *Dig Dis Sci* 1991;36:577–82.
- Fukuda Y. Suppression of *Helicobacter pylori* colonization with omeprazole. *Scand J Gastroenterol* 1996;31(Suppl 214):54–5.
- McGowan CC, Cover TL, Blaser MJ. The proton pump inhibitor omeprazole inhibits acid survival of *Helicobacter pylori* by a urease-independent mechanism. *Gastroenterology* 1994;107:1573–8.
- Bode G, Malfertheiner P, Ditschuneit H. Pathogenetic implications of ultrastructural findings in *Campylobacter pylori* related gastroduodenal disease. *Scand J Gastroenterol* 1988;23:25–39.
- Noach LA, Rolf TM, Tytgat GN. Electron microscopic study of association between *Helicobacter pylori* and gastric and duodenal mucosa. *J Clin Pathol* 1994;47:699–704.
- Fader RC, Davis CP. Effect of piliation on *Klebsiella pneumoniae* infection in rat bladders. *Infect Immun* 1980;30:554–61.
- Ogata T, Araki K. Electron microscopic study of the morphological changes of gastric mucous cell induced by *Helicobacter pylori* in human gastric ulcers. *J Submicrosc Cytol Pathol* 1996;28:255–64.
- Okada T, Adkins GF, Hori K, et al. Pathology associated with *Okadaella gastrococcus* infection among the young generation. *Gastroenterology* 2012;142 (5, Suppl 1): S-678(Mo1759).
- Okada T, Adkins G, Hori K, et al. *Okadaella gastrococcus* found in the stomach of *Helicobacter pylori*-positive young generation. In: Forbes K, El Omar E, Strachan N, Hold G, eds. *CHRO 2013 Abstract book: 17th International Workshop on Campylobacter, Helicobacter and Related Organisms*, 15–19 September 2013, Aberdeen, UK: the Society for General Microbiology. 2013:77(p64).
- Okada T, Adkins G, Hori K, et al. A modified alcian yellow and toluidine blue staining method to visualize intracellular *Okadaella gastrococcus*. *Gastroenterology* 2011;140(5, Suppl1):S-318(Sa1743).
- Okada T. *Okadaella gastrococcus*: The intracellular bacteria present in the classic carcinogenic cascades. *DDW 2011*. Chicago, IL. 2011. <http://www.youtube.com/channel/UCOaZhl0UCrI-vjSlxsvaDqQ> (accessed 5 Oct 2013).
- Okada T, Adkins G, Jones M, et al. The presence of *Okadaella gastrococcus* in the gastric erosive ulcers. *Am J Gastroenterol* 2003;98(9, Suppl):S43(123).
- Okada T, Adkins G, Bradley J. Pathogenesis of *Okadaella gastrococcus* associated gastric mucosal lesions. *J Clin Oncol* 2005;23(16S, Suppl):4262.
- Lee A. The microbiology and epidemiology of *Helicobacter pylori* infection. *Scand J Gastroenterol* 1994;29(Suppl 201):2–6.

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