Ex vivo visualization of Helicobacter pylori using an endocytoscopic probe

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ABSTRACT
Observation of microorganisms by endoscopy has been technically difficult. In this study, we tried to visualize bacteria during endoscopic examination to provide a powerful tool for a diagnosis of gastrointestinal infection. We observed the bacterium Helicobacter pylori, cultured ex vivo, using a novel ultra-high “magnified endoscopy” system (“endocytoscopy” prototype, Olympus Medical Systems). Cultures were prepared from gastric mucus obtained from three gastric ulcer patients. H. pylori in the supernatant of the culture medium were observed directly by endocytoscopy. Staphylococcus aureus and red blood cells were used as controls. H. pylori in the culture medium were observed directly by endocytoscopy, and recorded using a video recorder. Live, moving bacteria can be visualized and recorded ex vivo using this new “endocytoscopy” technology.

Helicobacter pylori is a bacterium found in the human digestive tract, especially the stomach and duodenum (9). It causes gastric and duodenal ulcers (2, 10) and gastric cancer (2, 10), and is strongly correlated with hematological diseases such as MALT lymphoma (2) and idiopathic thrombocytopenic purpura (1). Procedures developed for the detection and clinical diagnosis of H. pylori (8) include biopsy (3), bacterial culture, urease test (4, 11), and measurement of antibodies in serum and/or urine. Detecting IgG antibodies to H. pylori using Western blot method was proved to be useful for the accurate diagnosis of past infection (7). However, it has so far not been possible to observe live bacteria on mucous epithelia by endoscopy.

Recently, a new ultra-high magnifying technology—termed “endocytoscopy” or “magnified endoscopy”—was developed; it uses a flexible endoscope attached to a microscope to directly visualize mucous epithelial cells. This technology has been applied for in vivo pathological study of colon cancer (5). We used this endocytoscope to observe H. pylori in cultures prepared from the gastric mucus of ulcer patients (ex vivo study) as a preliminary step for future in vivo applications.

Three patients with gastric ulcer/chronic gastritis were enrolled in this study, all with approval by the local ethics committee. Gastric mucus was obtained from biopsy, with the patients’ written informed consent. The presence of H. pylori was confirmed by tissue culture, pathological study, and urea breath test. Bacteria were cultured using a semi-liquid agar culture medium specialized for H. pylori (Nissui Pharmaceutical Co., Tokyo, Japan), containing equine serum, soybean peptone, sodium chloride, and selective antibiotics. The agar concentration was 0.30%. Bacteria were incubated for five days at 37 °C and identified as H. pylori based on gram stain and urease activity. Aliquots (20 μL) of the culture medium were placed on glass slides and observed directly without any staining. Microscopic images of

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the bacteria were recorded at room temperature. The endocytoscope used was an Olympus XEC-120 system (prototype, Olympus Medical Systems Inc., Tokyo, Japan). It has a length of 250 cm and diameter of 5 mm, and is attached to a digital video monitor (14 × 14 inches) which allows magnifications up to x1100. The image from the scope is visible on a CRT monitor and recorded simultaneously on a digital video recorder (Sony, Tokyo, Japan).

Figs. 1A, B and C show *H. pylori* in a culture medium, as observed with the endocytoscope. Our short video of moving *H. pylori* is available on the Internet (6). The rod-shaped bacteria were moving in various directions, and spinning around by action of pili. The image was similar to that from a conventional microscope. Movement of the bacteria was observed for at least five minutes. We tried various recording conditions for optimal contrast and visibility. A reflective background surface (*e.g.*, silver-colored metal sheet) was required for clear visualization of the bacteria. Test tube clamps were used to fix the probe in a stable position.

As controls, we observed suspensions of cultured *Staphylococcus aureus* (Fig. 2), *Escherichia coli* (not shown), and normal red blood cells (Fig. 3) by the same procedure. No movements of *S. aureus* and *E. coli* in the culture medium were observed, in contrast to *H. pylori*. Characteristic morphological differences among the three bacterial species were seen.

This is the first report of use of endocytoscopy to observe living microorganisms. The technique was originally designed for *in vivo* observation of gastrointestinal epithelial cells without biopsy and has been applied for study of gastrointestinal cancer by Inoue et al. (5), who were able to distinguish benign vs. malignant lesions. We found that the endocytoscopy...
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Scope is suitable for the observation and video recording of live, moving bacteria in a culture medium, even without staining. Images of moving *H. pylori* were similar to those from conventional microscopes. The novel part of the system is the endocytoscopic probe; otherwise, the equipment is the same as that used for conventional endoscopy. Facilities set up for conventional endoscopy can readily adapt their equipment for endocytoscopy once the probes are widely available.

We tested many culture media and growing conditions to keep *H. pylori* alive and moving. The bacteria tended to grow near the surface of the culture medium, and along the test tube wall. At least four days of culture were necessary. Use of the semi-liquid culture medium as described was important; mobility and motility were less pronounced in liquid culture media often used for the selective culture of *H. pylori*.

This was a pilot *ex vivo* study. The future *in vivo* use of endocytoscopy (magnified endoscopy) has many obvious advantages. The technology can be used in narrow spaces such as the gastrointestinal and respiratory tracts. Bacteria or other microorganisms can be detected during endoscopic examination without waiting for pathological diagnosis. The patient can see the live, moving microorganism in his/her own body, which makes it easier to convince him/her of a *H. pylori* infection. The endoscopist can observe *H. pylori* on the epithelium *in vivo*, under specific conditions, e.g., antibiotic medication.

Studies on the *in vivo* use of endocytoscopy for the observation of bacteria on the human gastrointestinal mucous membrane are in progress. A novel endocytoscopy technology was applied for the clear *ex vivo* visualization of *H. pylori* in a culture medium. In the future, the technology will allow the *in vivo* observation of live, moving microorganisms on human mucous epithelia, even during routine endoscopy.

REFERENCES