ORIGINAL ARTICLE

Detection of Mycobacteria in Crohn's Disease by a Broad Spectrum Polymerase Chain Reaction

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Background: The role of mycobacterial infection, particularly related to *Mycobacterium avium* subsp *paratuberculosis* (*Map*), in Crohn's disease has long been debated. We developed primer pairs capable of detecting a broad spectrum of mycobacterium and employed them to investigate surgical specimens from patients with Crohn's disease.

Methods: Pan mycobacterium primers of the 65-kDa heat shock protein gene (*Hsp65*) were used in a polymerase chain reaction (PCR) to examine 12 surgically-resected, formalin-fixed, paraffin-embedded specimens from 11 patients with Crohn's disease. The DNA sequences of amplicons were aligned with those in GenBank.

Results: Mycobacterial DNA was found in specimens from three of 11 patients. *M. mucogenicum* was identified in a specimen from one patient and *M. tuberculosis* in two, but *Map* was not identified in any.

Conclusion: *Hsp65*-based PCR can be employed to search for occult mycobacterial infection of the gastrointestinal tract in patients with a diagnosis or suspicion of Crohn's disease. This approach may have a therapeutic implication. [*J Formos Med Assoc* 2006;105(4):290–298]

Key Words: Crohn's disease, heat shock protein gene, *Mycobacterium mucogenicum*, *Mycobacterium tuberculosis*, polymerase chain reaction

Crohn's disease was initially reported as chronic interstitial enteritis by Dalziel in 1913,[1] and then described as regional ileitis by Crohn in 1952.[2] It is an idiopathic inflammatory bowel disease characterized by noncaseating granulomas with transmural inflammation. However, the resemblance of Crohn's disease to paratuberculosis of ruminants (Johne's disease)[3,4] implies that *Mycobacterium avium* subsp *paratuberculosis* (*Map*) may cause Crohn's disease in humans. This hypothesis has prompted many investigators to determine if *Map* can be detected in Crohn's disease, particularly after the emergence of polymerase chain reaction (PCR)-based techniques. So far, at least 32 PCR-based studies of Crohn's disease have been reported. Among these, 18 studies have found *Map* DNA,[5–12] whereas 14 have reported negative results.[13–19] This discrepancy further adds to the complexity of an already confused pathogenesis of Crohn's disease.

Several possible explanations have been provided for the possible false-negative findings. First, sampling paucimicrobial tissue might yield a DNA extract that contains insufficient template for detection by PCR assay. Second, suboptimal procedures, such as inadequate disruption of bacterial walls and the presence of inhibitors for enzyme reactions, might result in detection failure.

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Third, some PCR products may contain too little DNA to be visualized unless sensitive methods such as hybridization with isotope-labeled probes or amplification by nested PCR are employed. It has been noted, however, that most of the reported PCR assays targeting the repetitive IS900 insertion element in the *Map* genome[20,21] were very sensitive, particularly when nested PCR was employed. Therefore, the negative or low yield of positive samples probably implies that no (or an insignificant percentage of) patients with Crohn's disease were infected with *Map*.

We speculated that the geographic variation of mycobacteria may provide an additional explanation for this discrepancy. There is no evidence to suggest that *Map* is the only species of mycobacterium that can cause Crohn's disease. In addition, Johne's disease is, in fact, morphologically similar to *M. avium* intracellular infection rather than to Crohn's disease.[22] To test this hypothesis, we first investigated whether the nucleotide sequence polymorphism of the mycobacterial cell wall 65-kDa heat shock protein gene (*Hsp65*) can distinguish a broad spectrum of mycobacterium, and then applied this technique to determine if mycobacteria other than *Map* could be detected in the surgical specimens of patients with Crohn's disease.

Methods

All patients with a diagnosis of Crohn's disease between 1992 and 2003 were identified from the files of the Department of Pathology, Mackay Memorial Hospital, Taipei. This study was conducted according to the guidelines of the hospital's institutional review board. The final study group comprised 12 specimens from 11 patients (Table 1). Each had typical features of Crohn's disease, including clinical presentation, imaging profiles, and histopathologic features. The histo-

Table 1. Summary of patients diagnosed with Crohn's disease

Case	Presentation, treatment and outcome
1	35-year-old female with right lower quadrant pain, a palpable mass and leukocytosis; treated by segmental resection of the cecum and terminal ileum; postoperative intestinal obstruction managed by exploratory laparotomy and lysis of adhesion
2	50-year-old male with ileus and a plain abdominal film showed small bowel dilation with gas retention
3	35-year-old female with intermittent right lower quadrant pain; computed tomography showed an appendiceal abscess with local extension into the right psoas-iliac muscles; treated by segmental resection
4	49-year-old female with chronic diarrhea for 2 years; treated by segmental resection of the small intestine and oral prednisolone; postoperative complications included enterocutaneous fistula, occasional diarrhea, malnutrition, and osteoporosis
5	41-year-old male with a 1-year history of chronic diarrhea, tarry stool and body weight loss; fistula formation in the resected small bowel
6	27-year-old male with a 1-month history of left lower quadrant pain and change in bowel habit; roentgenography showed paralytic ileus with segmental narrowing at the junction between the ileum and jejunum; treated by resection of the ileum
7	64-year-old male with abdominal discomfort initially treated by segmental resection of the cecum and ascending colon; persistent enterocolitis and complications (adhesions, fistulae) treated by numerous abdominal surgeries; diagnosis of Crohn's disease made after a right hemicolectomy during his third hospitalization; prednisolone prescribed intermittently
8	40-year-old male with hollow organ perforation; treated by sigmoid colectomy and ileal resection; died of pneumonia and sepsis
9	49-year-old male with intermittent abdominal pain; treated by partial colectomy, resection of the ileum and oral prednisolone
10	35-year-old male with a 4-year history of diarrhea; treated by right hemicolectomy; symptoms recurred 5 years later and treated by ileoileal bypass
11	22-year-old male with abdominal pain, vomiting and tarry stool; ileal resection for a perforation at the distal ileum; postoperative complications included functional bowel disturbance and constipation