**Background:** Little is known about the pathogenesis of Whipple disease, the reservoirs of *Tropheryma whippelii*, and the proportion of persons harboring the bacterium without "classic" intestinal abnormalities.

**Objective:** To assess the presence of *T. whippelii* in patients undergoing upper endoscopy for a variety of indications.

**Design:** Prospective and routine diagnostic examination of patients.

**Setting:** Three academic medical centers in California; Minnesota; and Heidelberg, Germany.

**Patients:** 342 patients undergoing endoscopy for evaluation of dyspepsia or possible peptic ulcer (group A, 173 patients), malabsorption (group B, 37 patients), or clinical suspicion of Whipple disease (group C, 132 patients).

**Measurements:** Small-intestinal biopsy specimens were tested by polymerase chain reaction for *T. whippelii* DNA and examined for histopathologic abnormalities.

**Results:** All patients with negative histologic findings also had negative results for *T. whippelii* DNA.

**Conclusions:** *T. whippelii* occurs only rarely in intestinal mucosa that lacks histopathologic evidence of Whipple disease. The human small intestinal mucosa is an unlikely reservoir for this organism.

Whipple disease is a systemic condition characterized by the presence of uniform rod-shaped bacteria in affected tissues and by macrophage inclusions that are positive on periodic acid–Schiff (PAS) staining. The classic clinical features of the disease include diarrhea with malabsorptive findings, abdominal pain, weight loss, arthralgias, lymphadenopathy, and occasionally, neurologic abnormalities. Numerous attempts to cultivate the causative bacterium in the past have failed. Molecular methods have revealed this organism, *Tropheryma whippelii*, to be a novel actinomycete (1, 2).

Recently, propagation in human fibroblasts has been reported (3), but this finding has not yet been reproduced by other investigators. Little is known about the pathogenesis of Whipple disease. Abnormalities of immune functions have been described (4) and are presumed to play a role as predisposing factors, but precisely defined preexisting immunologic defects have not been identified. Neither the natural habitats of the bacterium nor the route of infection are fully understood. Because of the frequency of intestinal manifestations, an oral route of acquisition is suspected (5, 6). The 16S ribosomal DNA (rRNA gene) sequence of the bacterium has been detected in a polymicrobial community of sewage effluent, suggesting a possible environmental habitat and source of infection (7). This possibility would be in accordance with the phylogeny of the bacterium (8) and with the epidemiologic features of the disease (5, 9).

Two recent reports have described polymerase chain reaction (PCR)–based detection of *T. whippelii* DNA in specimens of persons without the classic clinical or histologic features of Whipple disease. In one series, positive PCR results were obtained from saliva samples of 14 of 40 (35%) apparently healthy persons (10). In another series, positive PCR results were reported from intestinal biopsy or gastric juice samples of 14 of 105 (13%) patients undergoing elective endoscopy for reasons other than suspicion of Whipple disease (11). These investigators have speculated that *T. whippelii* is a commensal of the human gastrointestinal tract (10, 11). At the same time, several published series on diagnostic PCR have found no evidence of *T. whippelii* DNA in the intestinal biopsy specimens of controls (12–14).

These conflicting and confusing data and the treatable nature of this disease emphasize the need for additional information on the prevalence of *T. whippelii* in the intestinal mucosa of persons without evidence of Whipple disease by classic diagnostic methods, such as...
PAS staining or electron microscopy. Such information would provide further insights into the epidemiology and pathogenesis of the disease and would be crucial information with which to judge the indications for and interpretations of diagnostic PCR results. Thus, we examined intestinal biopsy specimens from three groups of patients. Two of the groups were prospectively examined: Patients underwent endoscopy for reasons other than Whipple disease in the first group and for work-up of malabsorption in the second group. In addition, we evaluated a third group—cases without histologic evidence for Whipple disease among intestinal biopsy specimens submitted to Stanford University (Stanford, California), the Mayo Clinic (Rochester, Minnesota), or the University of Heidelberg (Heidelberg, Germany) for diagnostic PCR testing for T. whippelii.

METHODS

Patients and Samples

Endoscopic biopsy specimens of the small intestine in patients from three categories were examined by using routine histologic methods, including PAS staining, and PCR testing for T. whippelii. Group A consisted of 173 patients who underwent upper gastrointestinal endoscopy for evaluation of dyspepsia, abdominal pain, or possible gastroesophageal reflux or peptic ulcer disease, without signs of malabsorption or consideration of Whipple disease. Group B consisted of 37 patients who underwent endoscopy in the work-up for malabsorption (endoscopy was prompted by unexplained weight loss or chronic diarrhea, for example, or by the referring gastroenterologist’s concern about protein or fat malabsorption). Biopsy specimens from groups A and B were obtained prospectively at the Mayo Clinic. Patients gave informed consent to allow additional specimens to be obtained for PCR testing for T. whippelii. Group C consisted of 37 patients who underwent endoscopy in the work-up for malabsorption (endoscopy was prompted by unexplained weight loss or chronic diarrhea, for example, or by the referring gastroenterologist’s concern about protein or fat malabsorption). Biopsy specimens from groups A and B were obtained prospectively at the Mayo Clinic. Patients gave informed consent to allow additional specimens to be obtained for PCR testing for T. whippelii. Group C consisted of 32 patients who underwent endoscopy for clinical suspicion of Whipple disease, based on intestinal or extraintestinal findings or both. The patients in group C were selected only if histologic studies (including PAS staining) of intestinal biopsy specimens gave no evidence of Whipple disease. The specimens in group C were submitted to the University of Heidelberg (70 specimens), Stanford University (30 specimens), and the Mayo Clinic (32 specimens). All biopsy specimens used in PCR analysis from groups A and B, as well as those from group C that were studied at the Mayo Clinic, were fresh-frozen samples; those from group C submitted to the University of Heidelberg and Stanford University were either fresh-frozen or formalin-fixed, paraffin-embedded specimens.

PCR Testing

The PCR assays performed at our institutions have been described previously in detail (2, 12, 13, 15). Briefly, assays were performed by using nonnested PCR protocols with an analytic sensitivity of 10 to 100 spiked copies of cloned T. whippelii 16S rDNA per reaction (12, 13, 15). The specificity of these assays has been verified by testing on a wide variety of other bacteria, including many closely related organisms and on control tissues from patients with other diseases (12, 13). Positive PCR results were confirmed by oligonucleotide hybridization or direct sequencing (12, 13, 15). In terms of diagnostic sensitivity, PCR testing gave positive results with histologically positive samples in 95 of 96 patients tested at our institutions. This series comprises 60 previously described patients (12, 13), as well as 36 previously unreported cases. Different rooms were used for pre- and post-PCR procedures at each of the three institutions. To assess the adequacy of DNA extraction and the presence of PCR inhibitors, all samples were tested by using PCR for human DNA (β-globin or c-myc), as described elsewhere (12, 13).

RESULTS

None of the intestinal biopsy specimens in groups A, B, and C showed histologic evidence of Whipple disease, as defined by previous histologic descriptions and criteria (5, 16). All specimens from the 173 patients in group A and the 37 patients in group B yielded negative PCR results for T. whippelii DNA (Table) and positive results for human DNA. In group C, all intestinal biopsy specimens from 70 patients tested at the University of Heidelberg, 30 patients tested at Stanford University, and 32 patients tested at the Mayo Clinic were negative for T. whippelii DNA (Table), and all were positive for human DNA. Symptoms in this group included diarrhea; arthritis; unexplained weight loss; and central nervous system signs, including cerebellar ataxia and features of hypothalamic disease. Included in
this group was one patient with cerebellar ataxia who had PCR-positive cerebrospinal fluid.

**DISCUSSION**

The distribution and abundance of the Whipple disease bacterium, *T. whippelii*, in nature remain poorly defined. Dependence on clinical recognition of this disease, and hence on its “typical” clinical features, may have led to biased and limited surveillance for this organism. Our data address this problem to some degree and allow an important comparison to be made with findings recently published by two groups that suggest a surprisingly high prevalence of *T. whippelii* DNA in the upper alimentary tract of persons without findings typical of Whipple disease (10, 11). We examined duodenal biopsy specimens from three different sets of patients. Group A represents patients with normal duodenal histologic findings who underwent endoscopy for reasons other than suspicion of Whipple disease and whose probability of harboring *T. whippelii* might be considered at most only slightly higher than that of the normal population. Groups B and C might be considered to have had a higher pretest probability of harboring *T. whippelii* because symptoms and clinical findings consisted of malabsorption, one of the cardinal symptoms of Whipple disease, or otherwise led to the inclusion of Whipple disease in the differential diagnosis.

No evidence of *T. whippelii* DNA was found in any of the intestinal biopsy specimens of the three groups in this study. It should be mentioned that a previously tested intestinal biopsy sample from a patient with negative intestinal histologic findings examined at Stanford University yielded a positive PCR result for *T. whippelii* DNA, as reported elsewhere (15). This patient had uveitis, and positive results were also obtained at the extraintestinal site (ocular vitreous fluid) by PAS staining, electron microscopy, and PCR. In addition, two other previously examined patients with suspected Whipple disease and negative intestinal histologic findings had PCR results positive for *T. whippelii* (13). One of these patients had suggestive histologic findings in a lymph node. Thus, the results of this study and previous work performed at our institutions offer several insights. First, *T. whippelii* DNA was not detected in the histologically negative intestinal mucosa of patients for whom Whipple disease was not considered (group A).

Second, Whipple disease was not a common diagnosis in patients with malabsorption (group B), despite the common occurrence of malabsorption in this disease and the importance of including Whipple disease in the differential diagnosis. Third, *T. whippelii* DNA may be detected in intestinal mucosa in the absence of histopathologic evidence, but the rate at which this was observed is very low, even in selected patients with increased pretest probability.

Our data provide no support for routine PCR-based *T. whippelii* testing of intestinal biopsy specimens when histologic testing fails to demonstrate features of this disease, even in patients for whom there is clinical suspicion. As concluded previously (12), PCR appears useful for confirming the results of intestinal histologic testing. On the other hand, the data from patients with extraintestinal Whipple disease suggest that specimens should be acquired from affected anatomic sites for further analysis, since extraintestinal infection is not always apparent from analysis of intestinal tract specimens (12). This is further illustrated by a report of two patients with arthritis whose intestinal biopsy specimens yielded negative results on both histologic examination and PCR testing for *T. whippelii* (17); positive results, both by PCR and histologic analysis, were obtained from synovial fluid and tissue. Another issue that must be considered is that the patchy distribution of Whipple disease in the intestinal tract (18) may lead to false-negative test results. Multiple biopsy specimens were subjected to PCR analysis and histologic examination from the patient with uveitis tested at Stanford University (15), and this may be warranted in all cases.

Our results are similar to those previously described, in which control specimens were included in studies involving diagnostic PCR testing for *T. whippelii* (12–

**Table. Results of Polymerase Chain Reaction Analysis for *Tropheryma whippelii* in Small-Intestinal Biopsy Samples**

<table>
<thead>
<tr>
<th>Patient Group (Study Design; Indication for Biopsy)</th>
<th>Participants</th>
<th>Positive Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (prospective; no malabsorption or consideration of Whipple disease)</td>
<td>173</td>
<td>0</td>
</tr>
<tr>
<td>Group B (prospective; work-up of malabsorption)</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>Group C (routine diagnostic; consideration of Whipple disease)</td>
<td>132</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>342</td>
<td>0</td>
</tr>
</tbody>
</table>
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14). However, our results appear to conflict with those described in a report by Ehrbar and colleagues (11), in which 5% of intestinal biopsy specimens from 105 patients undergoing elective endoscopy were positive on PCR testing for T. whippelii DNA (although the indications for endoscopy were not described in that published report) (11). The reasons for this apparent discrepancy are not clear. Potential explanatory factors may include the different sensitivity of the PCR tests and different tissue fixation conditions. However, both are unlikely to have accounted for our lower rates of PCR-positive specimens for several reasons: 1) The detection limit of our PCR assays (10 to 100 rRNA gene copies) is at the lower end of the range for other diagnostic PCR tests (19), 2) almost all tested cases (95 of 96) with histologic evidence of Whipple disease were found to be PCR positive, and 3) fixation issues would not apply to our analysis of specimens from groups A and B. Nested PCR, as used in the study by Ehrbar and colleagues (11), can be fraught with problems of cross-contamination. Another possible explanation for the discrepant findings between the studies could be geographic differences in the prevalence of T. whippelii in the environment, leading to different exposure rates, or differences in the inherent susceptibility of the study groups to colonization.

Taken together, our results indicate that T. whippelii DNA is rarely found in the intestinal mucosa of humans who have indications for upper endoscopy and is rarely found without histologic features of Whipple disease. Clearly, our results are relevant only to the small intestinal mucosa and do not necessarily apply to saliva and gastric fluid, in which more frequent positive PCR results have been reported by others (10, 11). However, if our findings can be corroborated and extended to additional prospectively acquired specimens, one could conclude that humans do not serve as a significant reservoir for T. whippelii and may be insufficient to maintain this organism in nature. This conclusion is consistent with the hypothesis that this bacterium resides in an environmental niche. Further investigation will be required to define the features of this habitat.

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