Diagnosis of Whipple Disease by Immunohistochemical Analysis

A Sensitive and Specific Method for the Detection of *Tropheryma whippelii* (the Whipple Bacillus) in Paraffin-Embedded Tissue

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Key Words: Whipple disease; *Tropheryma whippelii*; Immunohistochemistry

Abstract

Whipple disease is a rare systemic infection characterized clinically by diarrhea, fever, weight loss, arthralgia, malabsorption, and other systemic manifestations. The etiologic agent, *Tropheryma whippelii*, has been cultured only rarely. By using a polyclonal rabbit antibody produced against a cultured strain of *T. whippelii*, tissue sections from 18 patients with Whipple disease were studied. Specimens from patients with histologic mimics and other infections served as control specimens. Immunostaining was identified in all 18 patients. Granular immunostaining was observed similar to that in periodic acid–Schiff (PAS) stains. In 2 patients, immunostaining was identified in specimens negative by H&E and PAS stains. In 4 patients studied before and after antibiotic therapy, immunostaining was retained but diminished in intensity and quantity. Immunostaining was not identified in any control specimen. Immunohistochemical analysis is a sensitive and specific method for the diagnosis of Whipple disease in paraffin-embedded tissue and may provide new opportunities to investigate the pathogenesis of the infection.

Whipple disease is a rare systemic infection characterized clinically by diarrhea, fever, weight loss, malabsorption, arthralgia, and, in some instances, cardiac and central nervous system manifestations.1,2 The causative agent of Whipple disease, *Tropheryma whippelii*, is an obligate intracellular bacterium that only recently has been propagated in vitro.3-6 The diagnosis of Whipple disease usually is based on the demonstration of periodic acid–Schiff (PAS)-positive, diastase-resistant bacilli in tissue sections, most frequently of the small intestine.7-10 The diagnosis also may be confirmed by polymerase chain reaction (PCR) assay for 16S ribosomal RNA genes of *T. whippelii*11-14 or by demonstration of characteristic bacillary bodies by electron microscopy.9,15

In the present study, polyclonal rabbit antibody produced against an in vitro–cultivated strain of *T. whippelii*3 was examined in paraffin-embedded tissue sections from 18 patients with Whipple disease. In control specimens, the antibody also was applied to a variety of pathologic processes that may be clinical or histologic mimics of Whipple disease. The objective of the study was to determine whether immunohistochemical analysis with a specific *T. whippelii* antibody would be useful for identification, diagnosis, and, potentially, determination of the prognosis of Whipple disease.

Materials and Methods

Eight cases of Whipple disease were retrieved from the autopsy files of the Johns Hopkins Hospital, Baltimore, MD, from the period January 1907 to December 1989. Surgical specimens from 10 additional patients with Whipple disease
were retrieved from the surgical pathology files of the same institution from the period January 1984 to December 1999. The diagnosis of Whipple disease was based on accepted histopathologic criteria.\textsuperscript{7,10} The diagnosis of Whipple disease was confirmed previously in 10 of 18 patients by electron microscopy and in 2 of 18 by PCR assay. Clinical data obtained by chart review included age, sex, clinical manifestations, and history of antimicrobial therapy.

The specimens were formalin-fixed, paraffin-embedded, and routinely processed. Four-micrometer-thick sections were stained with H&E. Sections also were stained by either the PAS method or the PAS–alcian blue (pH 2) method. For immunohistochemical analysis, routinely processed paraffin-embedded tissue sections were cut at 4 \( \mu \)m and mounted on capillary gap positively charged slides. If paraffin blocks were not available, one of multiple tissue sections from original glass slides was destained and transferred to capillary gap positively charged slides. For immunohistochemical analysis, slides were deparaffinized in xylene and rehydrated with graded ethanols. Slides were transferred to trays containing sodium citrate buffer (diluted to 1x from 10x, HIER Buffer, Ventana Medical Systems, Tucson, AZ) and subjected to antigen retrieval by steaming for 20 minutes at 80°C. Slides then were cooled for 5 minutes and transferred for automated staining performed on the BioTek-Tech Mate 1000 (Ventana Medical Systems). Slides were incubated at room temperature for 45 minutes with the primary antibody, a polyclonal rabbit antibody produced against an in vitro–cultivated strain of \( T \) whipplei\textsuperscript{3} at a dilution of 1:10,000. The antibody was detected by the avidin–biotin complex method using the ChemMate Secondary Detection Kit (SDK605, Ventana Medical Systems). The chromogen was 3,3’-diaminobenzidine.

Sections were counterstained with hematoxylin and cover-slipped using permanent mounting medium. Normal tissue and tissue from patients with a variety of pathologic processes served as negative control specimens Table 1. In addition, acetone-fixed suspensions of bacteria (\textit{Escherichia coli}, \textit{Salmonella} species, \textit{Shigella sonnei}, \textit{Streptococcus pyogenes} [group A], \textit{Streptococcus agalactiae} [group B], \textit{Streptococcus} species group C, \textit{Streptococcus} species group D, \textit{Streptococcus} species group G, \textit{Streptococcus pneumoniae}, \textit{Citrobacter freundii}, and \textit{Klebsiella pneumoniae}) with reported antigenic cross-reactivity were tested by immunofluorescence using the polyclonal anti–\( T \) whipplei at the same dilution used for immunostaining.

Since PAS-stained tissues may not reflect an accurate distribution of bacteria for the various histologic findings, tissue sections were evaluated qualitatively and semiquantitatively for several histologic, histochemical, and immunohistochemical features Table 2. The semiquantitative analysis was graded as + if the feature was lacking in most microscopic fields or regions of interest, ++ if the feature was easily identified but not present uniformly in most microscopic fields or regions of interest, or +++ if the feature was frequently and uniformly present in high abundance in most microscopic fields or regions of interest.

### Results

#### Clinical Data

Specimens from 18 patients were examined. Patients ranged in age from 36 to 64 years (mean, 47 years). The male/female ratio was 6:1. A total of 60 sections from 8 autopsies and 23 surgical specimens were examined. The autopsy sections consisted of small intestine (n = 8), lymph node (n = 8), and brain (n = 2). The surgical specimens were from biopsies of small intestine (n = 17), lymph node (n = 5), and brain (n = 1). In addition, in 2 of the autopsy cases, sections from heart, lung, esophagus, stomach, liver, spleen, bladder, prostate, and bone marrow were evaluated.

Of the 18 patients, 3 had symptoms limited to the central nervous system. Pre–antimicrobial therapy and post–antimicrobial therapy specimens were available from 4 patients.

#### Table 1

<table>
<thead>
<tr>
<th>Site</th>
<th>Diagnosis (No. of Specimens Examined)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestine</td>
<td>Normal (5); \textit{Mycobacterium avium intracellulare} (5); \textit{Histoplasma capsulatum} (3); Crohn disease (6); celiac sprue (3)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>Normal (5); sarcoidosis (8); \textit{Mycobacterium tuberculosis} (3); Gaucher disease (3); Langerhans histiocytosis (3); \textit{H capsulatum} (2)</td>
</tr>
<tr>
<td>Brain</td>
<td>Normal (5); abscess (4); infarction (3); demyelinating disease (3); progressive multifocal leuкоencephalopathy (2)</td>
</tr>
<tr>
<td>Colon</td>
<td>Normal (5); rectal muciphages (8); malacoplakia (2)</td>
</tr>
<tr>
<td>Heart</td>
<td>Normal myocardium (3); normal valve (3); acute bacterial endocarditis (6)</td>
</tr>
<tr>
<td>Lung</td>
<td>Normal (3); \textit{M tuberculosis} (3); \textit{Aspergillus} species (3)</td>
</tr>
<tr>
<td>Spleen</td>
<td>Normal (3); Gaucher disease (2)</td>
</tr>
<tr>
<td>Liver</td>
<td>Normal (3); sarcoidosis (2)</td>
</tr>
<tr>
<td>Bladder</td>
<td>Normal (2); malacoplakia (3)</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Normal (2)</td>
</tr>
</tbody>
</table>
Routine Microscopy

Sections of small intestine were examined in specimens from 17 of 18 patients. The classic histologic features of Whipple disease, including numerous foamy macrophages in the lamina propria, club-shaped villi, and prominent dilated lymphatic spaces, were present in 14 of 17 cases (Table 2). On PAS staining, the macrophages were filled with PAS-positive granular material. No abnormalities were noted in the small intestine of 2 patients, each of whom had symptoms limited to the central nervous system.

Sections of lymph nodes showed a spectrum of changes (Table 2). Mesenteric lymph nodes showed the greatest degree of abnormality. The architecture of the mesenteric lymph nodes was distorted by large vacuolated spaces analogous to the dilated lymphatics seen in the lamina propria of the small intestine. Rare multinucleated giant cells were noted, usually adjacent to the vacuolated spaces. Foamy macrophages were much less conspicuous than in the small intestine. The number of cells with PAS-positive granules also was correspondingly fewer than in the small intestine. The normal nodal architecture was relatively well preserved in lymph nodes from extraintestinal sites. Histologically, these nodes were characterized by vague granulomatous inflammation with scattered clusters of foamy macrophages containing PAS-positive granular material.

Immunohistochemical Analysis

Specimens from all 18 patients with Whipple disease were reacted with the Whipple disease bacillus antibody (Table 2). The staining was granular and predominantly intracellular and corresponded to the foamy macrophages seen with H&E staining. The pattern of immunostaining in untreated patients was virtually identical to that seen with the PAS staining method. Rare extracellular staining was seen in the lamina propria of the small intestine. In untreated patients, the intensity of immunostaining varied little from case to case. The quantity of material staining varied among cases. In the majority of cases (16/18), the foamy macrophages were stuffed with bacilli. In 2 cases, relatively few immunoreactive bacilli were identified. In 2 patients, focal immunostaining was identified in small intestinal biopsy specimens that previously had been interpreted as negative by H&E and PAS staining methods. Both patients had symptoms limited to the central nervous system and subsequently were diagnosed with Whipple disease by brain biopsy. In 2 patients, focal immunostaining was identified in sections from myocardium, lung, esophagus, stomach, liver, spleen, bladder, prostate, and bone marrow.

In 4 patients, the initial diagnostic specimen and specimens obtained after initiation of antimicrobial therapy were available for study. The interval before initiation of therapy ranged from 2 months to 24 months. Immunostaining was retained after initiation of antibiotics in all 4 cases studied. The intensity and the quantity of staining were diminished on successive biopsies. The staining became less granular and more amorphous. In each case, the pattern of immunostaining was similar to the pattern seen with the PAS stain.
No immunostaining was detected in the normal or disease control specimens or clinical bacterial isolates.

**Discussion**

Owing to the previous inability to culture *T whipplei* by routine laboratory techniques, the diagnosis of Whipple disease usually is based on demonstration of intracellular PAS-positive, diastase-resistant bacillary particles in tissue sections, primarily of small intestine, lymph node, and brain. In tissue sections of the small intestine with classic histologic features, the diagnosis of Whipple disease is relatively straightforward with few histologic mimics. In extraintestinal sites, however, the diagnosis often is more difficult. The presence of PAS-positive material is not specific for Whipple disease, and confirmation by PCR assay or electron microscopy often is performed for definitive diagnosis. The use of immunohistochemical analysis provides an additional sensitive and specific diagnostic technique that can be easily performed in most laboratories.

This is the first antibody specifically directed against a cultivated strain of the bacterium *T whipplei*. Previous immunohistochemical and immunofluorescence studies have demonstrated cross-reactivity for the Whipple bacillus with antisera for various bacteria, most notably, *Streptococcus* groups A, B, and G and *Shigella* serotype B. Cross-reactivity of the antibody used in the present study was not identified at the dilution used for immunohistochemical analysis when a large panel of laboratory isolates, including *E coli*, *Salmonella* species, *S sonnei*, *S pyogenes* (group A), *S agalactiae* (group B), *Streptococcus* species (groups C, D, and G), *S pneumoniae*, *C freundii*, and *K pneumoniae*, was tested. Furthermore, the intracellular
location of *T. whipplei* on immunohistochemical staining would distinguish this pathogen from any demonstrating cross-reactivity.

In contrast with the PCR assay, immunohistochemical analysis provides direct visualization of the bacillus in tissue sections. Although PCR is increasingly used to diagnose Whipple disease, the prevalence of *T. whipplei* in healthy people is not fully known. Although negative results have been shown for *T. whipplei* by PCR assay in small intestinal biopsy specimens in control populations, others have demonstrated it to be present, and the prevalence of the bacterium in body fluids of healthy people is not well established. One study detected DNA for *T. whipplei* in the saliva of 35% of clinically healthy people. Moreover, PCR cannot be considered a “gold standard” for the diagnosis of Whipple disease because specificity varies with procedure.
and investigator, there is risk for DNA contamination, and it may be difficult to perform on paraffin sections.

In the small intestine, the histologic features of Whipple disease are easily recognized. The classic combination of foamy macrophages in the lamina propria, dilated lacteals, and large droplet lipid deposits usually is striking. The differential diagnosis is principally that of *Mycobacterium avium* intracellulare (MAI) infection. Stains for acid-fast organisms readily distinguish *M avium* intracellulare, and the causative bacterium also is easily cultured. *Histoplasma capsulatum* and *Rhodococcus equi* also may have similar histologic features. The oval budding yeasts that characterize *H capsulatum* generally are easily demonstrated by silver stains. Rarely seen outside the immunocompromised host, *R equi*, unlike *T whipplei*, is easily isolated on routine microbiology laboratory media.

In contrast with the small intestine, the histologic features of Whipple disease in extraintestinal sites can be exceedingly difficult to recognize, particularly if unsuspected clinically. Foamy macrophages frequently are fewer, as are PAS-positive cells. The histologic appearance of affected lymph nodes often is nonspecific granulomatous inflammation. In the brain, the histologic features of Whipple disease mimic a wide variety of infectious and inflammatory disorders. Typical features include perivascular aggregation of foamy PAS-positive macrophages in a setting of reactive gliosis. These features are, however, nonspecific. The differential diagnosis for Whipple disease in the central nervous system also includes macrophage-rich lesions such as demyelinating diseases, cerebral infarction, and a host of infectious diseases. Although the original isolate of *T whipplei* against which the polyclonal antibodies used in the present study were prepared was obtained from the blood of a patient with endocarditis, no cases of Whipple disease–associated endocarditis were examined in the present study.

The pattern of immunostaining observed corresponds to the presence of PAS-positive material, and undoubtedly the antigen is a component of the cell wall. The specificity of the antibody was tested in a wide variety of normal and diseased tissues and in a variety of bacteria suspected to contain similar antigenic constituents and was found to be highly specific at the dilution used. The systemic nature of Whipple disease is demonstrated by the presence of immunostaining in virtually every tissue sampled in 2 of the autopsy cases. Similar to the PAS staining method and electron microscopy, immunostaining was retained after initiation of antibiotic therapy, with a gradual reduction in quantity and intensity of staining, which differs from PCR results in which the bacterial antigen and intensity of immunostaining were observed to diminish in successive biopsy specimens from 4 patients studied after initiation of antibiotic therapy. Unlike electron microscopy, this technique cannot differentiate between persistence of viable organisms and bacteria in various stages of degeneration.

Immunohistochemical analysis for *T whipplei* is a sensitive and specific method for the diagnosis of Whipple disease that can be easily performed in most laboratories. This method offers added specificity over PCR methods owing to the direct visualization of bacilli and antigens within cells in tissue sections and offers increased sensitivity and specificity over the traditional PAS staining method. This technique may be useful for monitoring response to therapy. Potential applications of immunohistochemical analysis not addressed in the present study include use on cell-block or cytocentrifuge preparations of cerebrospinal fluid from patients suspected of having central nervous system involvement and in vitreous fluid and joint aspirates. In addition to its importance for diagnostic and therapeutic purposes, this method also offers new opportunities to investigate the pathogenesis of Whipple disease.

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Presented in part at the Annual Meeting of the United States and Canadian Academy of Pathology, Atlanta, GA, March 3-9, 2001.

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