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Whipple's Disease and *Tropheryma whippelii*: Secrets Slowly Revealed

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Whipple's disease was described in 1907 and was designated "intestinal lipodystrophy," despite the detection of bacteria in 1 specimen. This finding was later substantiated by the success of antibiotic therapy, which resulted in dramatic clinical responses, and by use of electron microscopy, which detected monomorphic bacilli in affected tissues. Many attempts at culture failed, and these bacteria were characterized as actinomycetes for the first time by means of broad-range 16S rDNA amplification and molecular phylogenetic methods. The name *"Tropheryma whippelii"* was proposed for this bacterium. Whipple's disease is a systemic disease that affects many organ systems, producing protean manifestations. This article summarizes recent developments with regard to this topic as well as unanswered questions regarding the pathogenesis and acquisition of infection, the biology and ecology of the organism, the clinical spectrum of disease, diagnosis of the disease, and therapy.

In 1907, George H. Whipple described autopsy findings for a 36-year-old patient who had a 5-year history of an illness that was dominated by arthritis, fever, chronic cough, weight loss, and diarrhea [1]. He observed deposits of fat and fatty acids in the intestinal mucosa and mesenteric lymph nodes; he assigned the term "intestinal lipodystrophy" to this disease. In 1961, bacteria were detected in affected tissues by means of electron microscopy [2, 3]. However, subsequent attempts to cultivate these bacteria failed. In the early 1990s, characterization on the basis of molecular phylogeny was achieved by means of broad-range bacterial rDNA PCR analysis [4, 5]. A newly acquired 16S rDNA sequence revealed a phylogenetic relationship between the bacterium and the actinomycetes, although there was no known close relative, and the name *Tropheryma whippelii* was proposed [5]. Whipple's disease is considered to be rare. In the only published monograph on this entity, Dobbins [6] compiled information from 696 cases available through 1986.

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CLINICAL MANIFESTATIONS

Whipple's disease is a systemic disease with a propensity for affecting the gastrointestinal tract [6]. Its clinical manifestations have been compiled and extensively discussed in several case series and reviews [6–11]. Intestinal manifestations are most commonly reported; these help to define what is known as "classical" Whipple's disease, which includes weight loss, diarrhea, and abdominal pain. Intestinal symptoms are often preceded by arthralgias for several years (up to 30 years). Abdominal and peripheral lymphadenopathies are also common.

Extraintestinal disease often involves the brain and the heart. Endocarditis, myocarditis, and pericarditis have all been reported. Symptomatic Whipple's disease of the CNS can occur at the time of initial diagnosis and can accompany intestinal manifestations, but it is more commonly reported as the cause of disease relapse during or after antibiotic treatment [6, 12]. Relapses in the CNS pose a serious challenge to clinical management, because they can be refractory to antibiotic treatment. Cases of primary neurological disease without detectable intestinal involvement have been reported [13, 14]. Ocular involvement (e.g., uveitis) has been reported, including cases in

patients who do not have grossly apparent intestinal disease [6, 15]. "Exotic," or rare, manifestations of Whipple's disease include prosthetic joint infection [16], spondylodiskitis [17], and extreme insomnia [18].

DIAGNOSIS

Histopathologic or cytological analysis by means of periodic acid–Schiff (PAS) staining are the standard methods used for diagnosis of Whipple's disease. The characteristic feature of the disease is the presence of macrophages with intracellular inclusions that react with the PAS stain and appear magenta (i.e., sickleform particle–containing cells), especially in the lamina propria of the small intestine. The inclusions reflect accumulations of degraded cell wall and intact bacteria. Electron microscopy has been recommended to confirm histopathologic diagnoses, especially in extraintestinal sites [6].

Diagnostic PCR assays for *T. whippelii* are increasingly being used to establish and confirm the diagnosis of Whipple's disease [19–24]. Although data on clinical sensitivity and specificity are scarce and difficult to acquire, well-optimized PCR assays are capable of detecting as few as 10 copies of the 16S rRNA gene per reaction, and PCR analysis of histologically positive specimens almost always yields positive results [19, 20]. There is still room for improvement of PCR testing and improvement in the selection of optimal specimen types. For example, not all PCR assays have been validated with thorough measurement of performance characteristics and identification of amplified products. An overview of diagnostic methods is given in table 1.

Despite the apparent rarity of Whipple's disease, the fact that it can occur in the absence of "classical" intestinal manifestations emphasizes the importance of considering the diagnosis in patients with atypical presentations. Certainly, Whipple's disease should be suspected in patients with weight loss, diarrhea, arthralgias, and abdominal pain or in patients with arthralgias, fever, and minor gastrointestinal complaints [6]. In these cases, upper gastrointestinal endoscopy is indicated, and several biopsy specimens from the lower duodenum should be obtained (because patchy disease involvement is possible) and subjected to histopathologic examination. In his monograph, Dobbins [6] provided a list of additional clinical settings in which Whipple's disease should be suspected. Dementia with no apparent cause, or chest pain and chronic cough with lung infiltrates that simulate sarcoidosis are examples of syndromes on this list. Enlarged intra-abdominal and peripheral lymph nodes that are hypodense on CT scans and hypoechoic on ultrasonograms, as well as skin hyperpigmentation that is not related to adrenal dysfunction or hyperbilirubinemia, may provide diagnostic hints [6]. In cases of suspected extraintestinal Whipple's disease, it is advisable to obtain specimens from the affected anatomic sites, in addition to intestinal biopsy specimens. This strategy applies to initial presentations with minimal or no apparent intestinal involvement [15, 27]; in addition, it applies to patients after treatment of Whipple's disease, when either clinical findings persist or new atypical presentations occur, as is illustrated by a case of extreme insomnia occurring 8 years after diagnosis and treatment of intestinal disease [18].

THERAPY AND MONITORING

There are still no randomized, double-blind trials of different antibiotic regimens upon which to base recommendations for the treatment of Whipple's disease. On the basis of the combined observations from many case reports [6], several patient series [9–11], and retrospective analyses [30, 31], the therapy most commonly associated with clinical success is initial iv treatment with penicillin G and streptomycin, or a third-generation cephalosporin, followed by administration of co-trimoxazole for at least 1 year (table 2). The main objectives of treatment of Whipple's disease are to eradicate primary (usually intestinal) disease and to prevent relapse. Considering the tro-

Table 1. Diagnostic methods for Whipple's disease.

Method	Sample types	Comments	Reference
Routine histologic analysis (with PAS staining)	Tissues (e.g., intestinal, brain, etc.)	Standard method; PAS-positive, diastase-resistant, non–acid-fast inclusions in macrophages are highly suggestive	[6, 25]
Cytological analysis (with PAS staining)	Body fluids (e.g., CSF, joint fluid, vitreous fluid)	Body fluids should be fresh (≤1 h)	[6, 15, 26, 27]
Electron microscopy	Tissues, body fluids	Recommended for confirmation of routine histopathologic analysis; time consuming; shows bacteria of typical size and shape	[6, 28, 29]
PCR analysis	Tissues, body fluids	Alternative test for confirmation; available in research and reference laboratories and few commercial laboratories	[5, 19–24]

NOTE. PAS, periodic acid–Schiff.

Table 2. Recommended therapy for Whipple's disease.

Timing	First choice	Alternative
Initially (first 10–14 days)	Pen G (6–24 million U iv q.d.) plus Stm (1 g im q.d.) or third-generation cephalosporin (e.g., Ctri 2 g iv q.d.)	TMP-SMZ (160 mg/800 mg po b.i.d.)
Long term (~1 year)	TMP-SMZ (160 mg/800 mg po b.i.d.)	Dox (100 mg po b.i.d, Cfix (400 mg po b.i.d.), or Pen V potassium (500 mg po q.i.d.)

NOTE. Dox, doxycycline; Cfix, cefixime; Ctri, ceftriaxone; Pen, penicillin; Stm, streptomycin; TMP-SMZ, trimethoprim-sulfamethoxazole.

pism of *T. whippelii* for the CNS and, thus, the threat posed by relapses in the CNS [26, 30], early use of drugs with good penetration of the blood-brain barrier is important.

Well-established protocols for patient follow-up during and after therapy are also lacking. Routine periodic assessment of sites of frequent disease involvement, such as the abdominal lymph nodes, cardiovascular system, and CNS, would be prudent. PAS-positive macrophages undergo morphological changes but persist for up to several years [25]; intact bacterial cells (with status determined by means of electron microscopy) are shorter lived, disappearing after a few months [28]. Positive results of PCR analysis of intestinal specimens (results indicate bacterial DNA) convert to negative results usually within 1–12 months after initiation of therapy [19].

One study suggested that PCR analysis of intestinal specimens may be useful for monitoring the efficacy of therapy [20], whereas another investigation found that some patients for whom the results of PCR analysis of intestinal samples are negative develop relapses in the CNS [19]. Cytological or PCR-based examinations of CSF may be useful for both initial assessment and monitoring of the development of CNS complications during therapy [26]. At present, there are no solid data that one can use to decide when antibiotic therapy should be terminated, but therapy with an overall duration of at least 1 year is considered necessary [30, 31]. The currently available data, albeit scant, suggest that posttreatment progression and relapse of disease are caused by the original infecting bacterial strain (see below), rather than by reinfection by a different strain.

IS WHIPPLE'S DISEASE UNDERDIAGNOSED?

The question of whether a disease or infection has been underdiagnosed naturally arises when new, more-sensitive diagnostic methods become available. Whipple's disease has traditionally been recognized by its "classical" clinical features. One would expect that this circumstance imposes a bias on the recognition and description of the full spectrum of disease manifestations. For example, Whipple's disease was retrospectively diagnosed in a specimen from 1895 that was stored at the Westminster Museum in London, by means of the newly available PAS stain [32]. The introduction of endoscopy in the 1970s, used in combination with PAS staining (which was introduced in the 1940s), led to the diagnosis of cases with intestinal pathology but "atypical" symptoms. By use of other diagnostic procedures (e.g., radiographically guided tissue biopsy or PCR analysis), cases of extraintestinal disease accompanied by minimal or no apparent intestinal involvement have been diagnosed [15, 17, 27, 33]. These diagnosed cases might have been missed before the availability of these methods.

At the same time, Whipple's disease is considered to be invariably fatal when it is not treated with antibiotics [6]. If a significant number of cases were unrecognized and untreated, one might expect some of them to be discovered at autopsy; however, this is not a common event. It is possible that spontaneous remission or resolution of disease occurs, and it is conceivable that patients with unsuspected disease are cured when they undergo short courses of antibiotic therapy for other complaints. In this context, the observation by Fleming et al. [9] of a case of long-term remission after a 5-day course of antibiotic therapy is intriguing. Furthermore, it has been suspected that the frequent use of antibiotics in general medical practice, in dosages and durations inadequate for cure, may have altered the age of presentation with Whipple's disease during the past several decades [34].

Overall, it seems unlikely that a significant number of advanced cases of Whipple's disease go undiagnosed; however, it remains unclear whether this theory holds true for less severe cases and for those that may be cured by short courses of antibiotics. Over a period of 30 years, Dobbins [6] noted a relatively stable incidence of Whipple's disease, with a ratio of 1 published case to every 4 unpublished cases. Given how little we know about the natural habitat of the organism (see the Pathogenesis and Acquisition of Infection section) and the route(s) of transmission to humans, it is even more unclear whether asymptomatic, transient, or persistent infections in privileged anatomic compartments (those that are usually free of microorganisms) are common occurrences.

MICROBIOLOGY

Electron microscopy of tissue specimens from patients with Whipple's disease reveals uniformity in bacterial size (0.2–

0.25 × 1–2.5 μm) and shape [6, 29]. The bacteria are surrounded by an unusual outer membrane not found in other gram-positive bacteria and unlike those seen in gram-negative bacteria: it appears to lack lipopolysaccharide. Some investigators have concluded that this membrane may be of host origin [29]. Phylogenetic analysis based on 16S rDNA sequence amplification with broad-range bacterial PCR primers revealed, for the first time, that the bacterium is a member of the actinomycetes [4, 5]; a subsequent analysis performed 4 years later, in which an expanded 16S rRNA sequence database was used, placed the organism between the genus *Cellulomonas* and a rare group of actinomycetes with group B peptidoglycan [35], with relatively distant relationships (16S rRNA similarity, 91%–92%) to members in either group. As a result, the lack of a known close relative prevents meaningful inferences of physiology and function for *T. whippelii* from well-studied cultivated members of this bacterial division.

Attempts to cultivate the Whipple's disease bacterium have had a troubled history; many attempts have been undertaken, and the "successful" isolation of a causative agent in a number of reports turned out to be nonreproducible [6]. One notable report by Schoedon et al. [36] was published in 1997. Heart valve tissue specimens obtained from 2 infected patients were inoculated onto human macrophages that had been treated with IL-4 in cell culture. This treatment impairs the microbicidal killing mechanisms of macrophages and facilitates the growth of intracellular microorganisms. Accumulation of PAS-positive intracellular inclusions and the persistence of PCR-amplified product after cell passage were interpreted as being indicative of growth of *T. whippelii*. However, these results have not been reproduced by other researchers (Maiwald and Relman, unpublished data; [37]). A nonvalidated PCR assay was used by Schoedon et al. [36], and the PAS reagent stains bacterial cell wall components even in advanced stages of degradation [6, 25].

Raoult et al. [38] reported another promising set of findings. An aortic valve tissue sample from a patient with endocarditis was inoculated onto a human fibroblast cell line, without special pretreatment of the cells (e.g., deactivation by cytokines). After 65 days of incubation, a cytopathic effect was observed, and microorganisms were seen by means of several staining procedures, including PAS staining. Fibroblast culture material was passaged 7 times, and after 285 days, a 3750-cm^2 infected cell monolayer was obtained from an initial inoculum of 1 cm^2 of cells. Several stains showed bacteria, and results of PCR analysis were positive for *T. whippelii* after each passage. The doubling time of the bacteria was estimated to be 18 days under these particular growth conditions, which is slower than that of *Mycobacterium leprae* in a mouse model (12 days). Immunofluorescence staining that used samples of the patient's serum as well as murine polyclonal antibodies raised against cultured material revealed bacteria in and on fibroblasts and in the original heart valve. Serological tests were also performed using cultured material as antigen. Elevated titers of IgM antibody were detected in 7 of 9 serum samples from different patients with Whipple's disease and in 3 of 40 serum samples from controls, whereas titers of IgG antibody were elevated for all 9 patients with Whipple's disease and for 29 of 40 controls.

Taken together, there is good evidence that these investigators have propagated *T. whippelii* ex vivo. However, the story is not yet complete: there is no documentation, by use of a quantitative method (e.g., quantitative PCR analysis), of an increase in bacterial numbers; this propagated organism did not originate from a patient with typical or "classical" Whipple's disease; and nothing is currently known about whether the described culture conditions reflect the optimal growth conditions for this organism. Although the reported doubling time renders this culture method impractical for routine laboratories, this report may constitute an important step toward the ultimate goal of routine propagation of *T. whippelii* in the laboratory.

Basic epidemiological tasks, such as tracking routes of infection and determining linkage between cases, require bacterial strain identification and discrimination. The first step toward strain typing of *T. whippelii* has been achieved using the bacterial 16S–23S rRNA intergenic spacer sequence. This sequence was initially determined from a specimen from 1 patient with Whipple's disease [35]; variability of the spacer sequence was addressed in subsequent studies [39–41]. One study [39] found homogeneity in the spacer sequences in 9 Swiss individuals; another study by the same group of investigators [40] found 3 different spacer types in 28 individuals whose geographic locations were not specified. A third study [41] found 5 different spacer types in 56 specimens from 43 patients from 4 countries; this study described the most common types, "1" and "2," in a similar ratio (~1:2) in patients from the United States, Germany, and Switzerland. Specimens from different anatomic sites generally yielded the same spacer types in individual patients, which supports the concept of systemic dissemination of a single bacterial clone [41]. However, 1 intestinal biopsy sample from 1 patient contained 2 sequence types, which raised, for the first time, the possibility of double infection with *T. whippelii* [41]. Despite these efforts, the 16S–23S rRNA intergenic spacer sequence with its 6 known variant types may not be adequate for discrimination between *T. whippelii* strains at a clinically relevant level. A more variable genetic locus or set of loci needs to be identified for this purpose.

PATHOGENESIS AND ACQUISITION OF INFECTION

Important unresolved issues pertaining to the pathogenesis of Whipple's disease include the source and route of infection and the possibility of differential host susceptibility. Very little is

presently known regarding these issues. Because of the prominence of intestinal manifestations, an oral route of acquisition is assumed [6]. The highest concentration of visible bacilli is usually found within the lamina propria of the small intestine, subjacent to the epithelial basement membrane [28]. It seems likely that bacilli translocate across or between the epithelial cells from the luminal to the basal zone, cross the basement membrane, and then elicit a macrophage-predominant response. Despite the recent report describing the propagation of *T. whippelii* in vitro with use of eukaryotic cells [38], intact and dividing bacteria are most often found in vivo outside of host cells [29]. Thus, this microbe may actually be an extracellular pathogen, and the keys to its optimal cultivation may be found within the microenvironment of the lamina propria.

Transient (during active disease) as well as persistent (after therapy) abnormalities of immune function have been described in patients with Whipple's disease [6, 42]; the persistent abnormalities are presumed to serve as predisposing factors. However, precise immune defects have not been adequately defined. The notion of preexisting host impairment is supported by the observation of opportunistic infections in some patients with Whipple's disease [43]. One patient appears to have benefited from adjuvant IFN-γ treatment [44]. *T. whippelii* DNA has also been detected in a patient with AIDS [45]. However, the issue is complicated by the common occurrence of malabsorption and malnutrition in patients with AIDS and their consequences for immune cell function. Further reports on the detection of *T. whippelii* in patients with AIDS have not appeared, but detection of this bacterium may have been missed by routine diagnostic examinations. Taken together, if there is a host genetic defect, the phenotype is relatively subtle.

Two recent publications reported PCR-based detection of *T. whippelii* DNA in specimens from persons with no signs of Whipple's disease. In one series, results of tests of saliva samples from 14 (35%) of 40 apparently healthy persons were positive [46] for *T. whippelii;* in another series, results of PCR analysis of intestinal biopsy or gastric juice samples were positive for 14 (13%) of 105 patients undergoing endoscopy for reasons other than suspected Whipple's disease [47]. These investigators speculated that *T. whippelii* is a commensal of the normal human gastrointestinal tract. On the other hand, several published series found no evidence for *T. whippelii* DNA in control intestinal biopsy specimens by use of PCR analysis [19–22]. Although additional data on *T. whippelii* DNA in saliva are not available, combined results from several institutions would argue against the human small intestine being a significant reservoir for *T. whippelii* [48].

In the analysis by Dobbins [6], farmers and carpenters were the professional groups most commonly affected by Whipple's disease. An epidemiological study in Germany [34] found a relatively homogeneous geographic and temporal distribution of cases. Most of the known phylogenetic relatives of *T. whippelii*, especially those on closer branches of the evolutionary "tree," are environmental organisms or plant pathogens [35]. These features point to a potential environmental habitat for *T. whippelii* and to the source of infection. Indeed, the results of a PCR-based search in 5 different sewage treatment plants, representing rich polymicrobial communities outside the human host, revealed that 25 of 38 samples were positive for *T. whippelii* [49].

CONCLUSIONS AND PERSPECTIVES

The Whipple's disease bacterium—recalcitrant to cultivation, encased by a thick and unique cell wall, and without known close relatives—has been slow to reveal its secrets. However, the past 10 years have been marked by a number of important findings. The organism has been identified and characterized at a molecular level, and a reliable diagnostic signature has been defined [4, 5]. The first stages of a bacterial typing scheme have been established [35, 40, 41]. A recent report suggests that propagation of the bacterium in vitro may be possible [38]. From either a propagated organism in ex vivo culture or by use of broad-range amplification methods with clinical specimens, we are certain to acquire a great deal of additional genotypic and phenotypic information about this bacterium during the next 5 years, leading to tools for serological diagnosis, development of new therapeutics, and insights into disease pathogenesis.

Genomics and a rapidly accumulating set of associated techniques are likely to yield a more complete genomewide perspective on the capabilities, gene responses, and deficiencies of this bacillus. As has been the case with other actinomycetes, we can expect to find unusual metabolic pathways, biosynthetic products of relevance to virulence (and of possible use as novel drugs), and clues about its natural environment. Sensitive and specific detection methods that are currently available can and will be used to define the preferred habitats of *T. whippelii* within and outside of the human host. For example, fluorescent in situ hybridization techniques can be used to map the anatomic distribution of *T. whippelii* rRNA in affected tissues. As observed with *Legionella pneumophila,* initial laboratory growth conditions (charcoal-yeast extract agar) may prove to be quite distinct from those that the organism has selected in the natural world (within free-living amoebae). Finally, a wide variety of tools and data sets will permit a reassessment of host susceptibility to Whipple's disease.

The Human Genome Project, by facilitating comprehensive surveys of host gene polymorphisms and variant gene responses, will provide significant contributions to the study of infectious disorders such as Whipple's disease, for which relevant laboratory models of disease are unavailable. It is a safe

bet that, during the next 10 years, many of the remaining mysteries about this disease and disease agent will be explained, and, with these explanations, profoundly important biological principles will be established

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