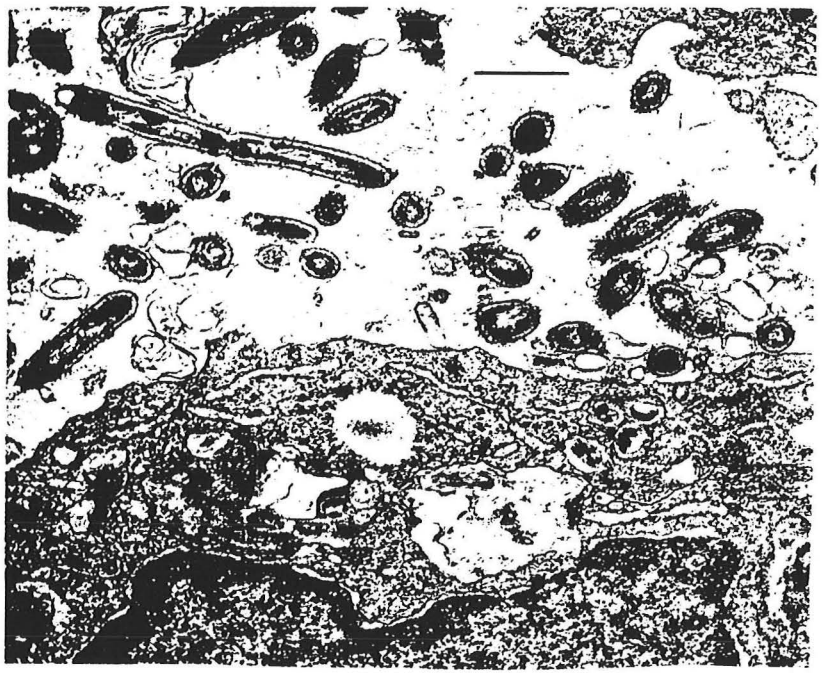
WHIPPLE'S DISEASE



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September 17, 1998

Whipple's disease continues to fascinate clinicians because of its varied manifestations which often make the diagnosis difficult and elusive[1]. The onset of disease is typically insidious and it may present without gastrointestinal symptoms and primarily affect other organ systems such as joints, heart, or CNS[2]. The disease is rare, with only about 800 published cases in the world literature[3]. In addition, the disease has a strange predilection for middle-aged, Caucasian men. The variety of presentations and the rarity of the disease are the main reasons that diagnosis is often delayed for years with sometimes detrimental or fatal consequences.

It has been known for more than forty years that Whipple's disease responds to antibiotic treatment and the Whipple bacillus was characterized morphologically by EM in the early '60's. However, the bacillus has resisted multiple attempts of cultivation and characterization by numerous investigators over the past several decades. It is only very recently that progress in this field has been made. The Whipple bacillus has finally been classified taxonomically as an actinomycete with the use of a novel PCR-based technique and it has been successfully cultivated *in vitro* utilizing an ingenious culture condition[4,5].

Thus, the purpose of these grand rounds is to:

1. review the clinical presentation of patients with Whipple's disease and its principal organ manifestations;

2. introduce the molecular biologic approach to bacterial classification with Whipple bacillus as an example;

3. outline the approach taken to finally cultivate the bacillus;

4. discuss possible immunological defects in patients with Whipple's disease; and

5. review the current recommendations for antibiotic treatment.

Historical Aspects

George Whipple was an instructor in pathology at Johns Hopkins University when he published his classical case report in 1907 of 'a hitherto undescribed disease' [6]. The patient was a 36 year-old physician who had died after a prolonged illness characterized by chronic cough, migratory arthralgia, weight loss, diarrhea, and fever. On admission, he had peripheral adenopathy, an abdominal mass and a swollen left ankle. Laboratory tests showed anemia and steatorrhea. He eventually underwent a laparotomy and the major finding was very enlarged and hard mesenteric glands.

The original patient

36 y/o white man-medical missionary

Chronic cough (5 yrs), migratory arthralgia (4 yrs),fever,weight loss and diarrhea (1yr)

PE :Adenopathy,abdominal mass and swollen ankle.

Labs :Anemia,steatorrhea

Expl.lap :Enlarged mesenteric glands

Presumptive dx : Tb vs Hodgkin's disease

Autopsy findings

Dilated jejunum with thickened wall

Enlarged mesenteric lymph nodes

Pleural,pericardial & peritoneal adhesions, aortic valve vegetation

Micr:Shortened villi in the jejunum,accumulation of fat & foamy cells in lamina propria and in lymph nodes

Silver stain : 'rod-shaped organisms (?)'

The presumptive diagnosis was tuberculosis or Hodgkin's disease of mesenteric glands. He died a few days later presumably from congestive heart failure. At autopsy, the jejunum was dilated with a thickened wall and the mucosa had multiple small yellowish grains. Microscopic examination of the jejunum showed shortened, broad-based villi with dilated, fat-filled lymphatics and the lamina propria was filled with large, mononuclear foamy cells(macrophages). Similar changes were observed in the submucosa. The mesenteric lymph nodes were enlarged and also filled with fat deposits and foamy macrophages. Additional findings included extensive pleural, pericardial and peritoneal adhesions and a small vegetation on the aortic valve. It is of interest that Whipple used a silver stain on a lymph node and observed 'great numbers of a rod-shaped organism (?).' They were found in the foamy macrophages and were thought to most closely resemble the tubercle bacillus. Whipple stated, 'whether this is the active agent in this peculiar pathological complex cannot be determined from the study of this single case, but its distribution in the glands is very suggestive.' Despite this astute observation Whipple suggested that the cause of the disease was 'some obscure disease of fat metabolism' and suggested the descriptive term 'intestinal lipodystrophy' as a name for the disease.

Only a limited number of case reports on intestinal lipodystrophy were published during the first half of this century and a number of other names for this disease were proposed such as 'intestinal lipogranulomatosis,' 'mesenteric chyladenectasis,' and 'steatorrhea arthropericarditica'[7,8]. The disease was uniformly fatal in that era. Since the early '50's, the name Whipple's disease has been the accepted name. In 1949, it was shown that the foamy macrophages in the intestine and lymph nodes of Whipple's patients stained positive for glycoproteins with the periodic acid-Schiff (PAS) stain, and the demonstration of PAS positive macrophages became the accepted diagnostic criterion for diagnosis[9]. In 1954, a critical review of the world literature revealed a total of 54 acceptable cases[10]. More than ninety per cent of the reported patients were males and most cases presented between the ages of 30 and 60 years.

Whipple's Disease 1.Series

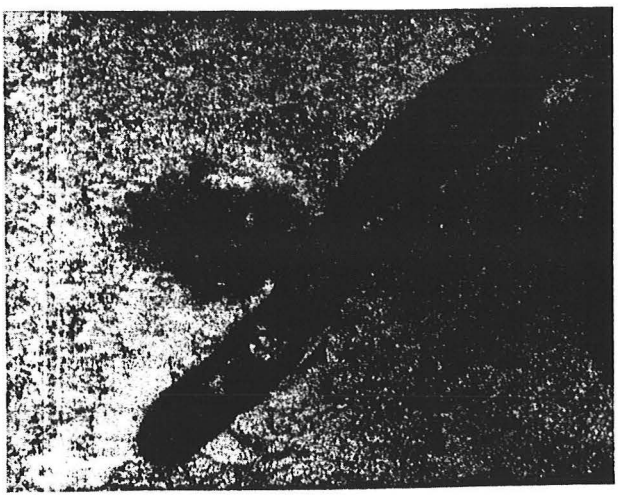
54 patients

Presenting symptoms		Physical findings	
• Diarrhea	94%	• Abdom.distension	87%
• Weight loss	93%	• Hypotension	74%
• Abdominal pain	87%	• Hyperpigmentation	56%
• Arthralgia	67%	• Abdom.mass	46%

Ann Intern Med 1954

The major symptoms were diarrhea (94%), weight loss (93%), abdominal pain and distention (87%), and arthralgia (67%). The important physical findings were abdominal distention (87%), hypotension (74%), skin pigmentation (56%), and abdominal mass(46%). Laboratory tests showed anemia (83%) and steatorrhea in 20 cases (not determined in 30 cases). Forty-four of the 54 cases had died from Whipple's disease and the diagnosis was first established at autopsy. Autopsy findings included polyserositis (pericarditis, pleuritis, and peritonitis) in 31 (56%) and cardiac valve vegetations in 14 (26%) which are probably underestimates of the true prevalence as these findings were not reported in a number of case reports. The etiology was still unknown but the frequent findings of arthralgia and polyserositis led investigators to believe that Whipple's disease was a collagen vascular disease and treatment with ACTH or corticosteroids was attempted with dramatic improvement in some patients. However, Paulley at that time had already published a case report where the diagnosis of Whipple's was made from jejunal and lymph node biopsies obtained at laparotomy[11] . The patient was fortuitously treated with two brief courses of chloramphenicol and made a full recovery. Paulley is credited with being the first to treat Whipple's disease with antibiotics although it was the patient's house physician and not Paulley who initiated the treatment with chloramphenicol. Paulley attributed the favorable response to an eradication of a secondary infection of the jejunal mucosa. In another case report from the early '50's, where again the diagnosis was made at exploratory laparotomy, treatment with two courses of penicillin was deliberately initiated in the belief that Whipple's disease was an infectious process[8]. The patient made a full recovery and remained well over a three year follow-up. These two case reports, however, remained unnoticed for several years.

It is curious that Whipple's original observation of 'rod-shaped organisms' in a lymph node was largely ignored up until 1961 when two groups of investigators independently reported their findings by light microscopy of gram positive bacilliform structures in the lamina propria of a Whipple's patient[12,13].



Both reports included electron microscopic examination and showed numerous elongated bodies in the lamina propria having an appearance characteristic of bacteria. One of the reports originated from this institution and the authors argued that the characteristic shape and gram-positive staining strongly suggest that the structures are indeed bacteria and that they may play a primary role in the etiology of the disease. These reports firmly proved the presence of gram-positive bacteria in the lamina propria of Whipple's patients and have since been confirmed by many light and electronmicroscopic studies of biopsies from multiple organs in patients with Whipple's disease. Subsequently, EM demonstration of bacillary structures became the gold standard for diagnosis of Whipple's disease. In addition, treatment with antibiotics became standard and the use of corticosteroids was abandoned.

Clinical Presentation

The typical presentation of a patient with Whipple's disease is that of a middle-aged, white man who has had migratory arthralgia for years and then develops diarrhea, weight loss, and abdominal pain. The presenting symptoms in a series of 126 patients with Whipple's disease published in 1970 are shown in the table [14]. 113 (90%) of the 126 patients were males and 122 (97%) were Caucasians. The mean age at presentation was 49 years with a range of 20 to 67 years. Hypotension was seen in about two-thirds of the patients and lymphadenopathy, hyperpigmentation, and abdominal tenderness in about one-half. The presence of occult blood in the stools was only tested in 36 patients but was positive in 29 (81%). Only eight patients presented with CNS abnormalities(6%). The most common finding on laboratory examination was the presence of anemia in 90% of the patients. Measurement of stool fat excretion was only performed in 46 patients and 43 (93%) had steatorrhea with a mean stool fat excretion of 23.6 g. Malabsorption of d-xylose was found in 25 of 32 patients tested (78%).

Whipple's disease -2. Series

(n = 126 ,1950 - 1969)
Caucasians 97 % ; males 90 %

Symptoms (%)		Physical findings(%)	
• Weight loss	95	• Hypotension	70
• Diarrhea	78	• Adenopathy	52
• Arthralgia	65	• Abd.tenderness	48
• Abdom pain	60	• Hyperpigment.	47
• CNS	6	• Fever	38
		• Occ.blood (36)	81

Two more recent reviews, one from the Mayo Clinic with 29 patients, and one from France with 52 patients, present very similar observations in terms of male predominance, ethnicity, age at presentation, and presenting symptoms and findings[15,16]. The only exception is a higher proportion of the patients presented with CNS symptoms, 43% and 21%, respectively.

Gastrointestinal manifestations

The common presentation with weight loss, steatorrhea, abdominal pain and, sometimes, a palpable mass, is caused by the infiltration of the lamina propria of the small intestine and mesenteric lymph nodes with bacteria-filled macrophages. The abdominal pain is usually diffuse and non-specific and may be accentuated by meals. The palpable abdominal mass when present has in many cases been shown by exploratory laparotomy to be enlarged mesenteric lymph nodes as it was in Whipple's original patient. Weight loss, diarrhea, and steatorrhea are the typical symptoms of malabsorption. The pathogenesis of malabsorption in Whipple's disease is probably multifactorial. The massive infiltration of the lamina propria by macrophages causes a flattening of the mucosal surface with blunted, broad based villi, and, as a result, a loss of absorptive surface area. The intestinal epithelial cells are cuboidal rather than columnar with sparse microvilli, and the cells are invaded by Whipple bacilli, which may result in epithelial cell dysfunction. The decrease in absorptive surface area and epithelial cell dysfunction are likely the cause of d-xylose malabsorption in these patients. Blockage of lymphatic flow from the intestine may contribute to fat malabsorption. The infiltration of the mesenteric lymph nodes by macrophages and fat causes an obstruction in lymph flow as evidenced by dilated central lacteals in the lamina propria and dilated lymphatic vessels in the mesentery commonly observed during exploratory laparotomy. Dietary fatty acids are resynthesized to triglycerides and incorporated into chylomicrons in the intestinal epithelial cells before export into the interstitial space in the lamina propria. Chylomicrons then diffuse into the central lacteals and are transported by the lymphatics to the blood. The lamina propria is packed with chylomicrons in patients with Whipple's

disease which further substantiates the fact that lymph flow from the lamina propria is impeded by lymphatic obstruction. Fat malabsorption is typically moderate with stool fat excretion in the range of 20 to 30 g/day. Hypoalbuminemia is another common feature in these patients and may be caused by amino acid malabsorption and protein-losing enteropathy. Only a few studies have documented the presence of intestinal protein loss[17]. The mechanism of intestinal protein loss may be secondary to lymphatic obstruction, as about 25% of the circulating plasma protein pool is filtered into the interstitial space and returned via the lymphatics to the blood. The common finding of occult gastrointestinal bleeding in Whipple's disease may be due to mucosal ulcerations and friability of the mucosal surface which has been reported in endoscopic studies[18,19].

Arthralgia

Migratory arthralgia often precedes the intestinal manifestations of Whipple's disease by several years. The most commonly affected joints are the knees, ankles and wrists[20]. The hip and smaller joints are rarely affected. The pain usually lasts hours to days without objective joint changes. However, arthralgia is associated with constitutional symptoms such as night sweats and fever in more than 50% of the cases. While the sedimentation rate is often elevated, other rheumatologic tests are negative and the joint manifestations are characterized as a seronegative arthritis. Occasionally, the joints can become warm, red, and swollen with an effusion as was the case of Whipple's original patient. PAS positive macrophages with bacillary bodies by EM have been demonstrated in the synovium in a few cases. The joint symptoms respond rapidly to antibiotic treatment. Many authors argue that Whipple's disease should be included in the differential diagnosis when a middle-aged, white man presents with seronegative arthritis.

Heart Disease

The true prevalence of cardiac involvement in Whipple's disease is not known.

Heart manifestations

(Autopsy study 1975;n=19)

Incidence (%)

- Physical abnl(murmurs,Ekg) 56
- Pericarditis 79
- Thickened MV 56
- PAS pos macrophages 100

In an autopsy study of 19 patients with Whipple's disease, it was found that 10 (56%) had clinical findings (murmurs, friction rub, EKG changes), 15 (79%) had adhesive pericarditis and 10 (56%) had a thickened, deformed mitral valve[21]. PAS positive macrophages were found in all 19 hearts in the valves, myocardium and pericardium. Apparently, none of these 19 patients had symptomatic heart disease despite these findings which underscores the fact that organ involvement can remain silent. However, there are case reports of patients presenting with aortic insufficiency[22], constrictive pericarditis[23,24], or myocarditis[25,26,27] in unsuspected Whipple's disease or in patients whose gastrointestinal symptoms had responded to antibiotic treatment. There are only a few case reports where cardiac involvement was documented during life and where the cardiac symptoms improved with antibiotic treatment[28,29]. The Whipple bacillus may also invade the coronary arteries causing an arteritis with luminal narrowing. The bacilli are predominantly found in the tunica media[30].

Lung Disease

Whipple's patient had a long history of chronic cough and autopsy showed pleural adhesions. In the old series of 54 patients published in 1954 where most patients died and underwent autopsy, only 14 patients reported chronic cough but 31 (60%) had pleural adhesions, which again emphasizes that lung involvement, just as cardiac involvement, can remain subclinical[10]. Sieracki performed extensive autopsy studies in five patients with Whipple's disease, and found PAS-positive macrophages in the interalveolar septa, connective tissue, and pleura in all five patients[31]. Symmons has reported on four patients with Whipple's disease where the pulmonary symptoms, chronic cough, shortness of breath, and pleuritic chest pain, preceded the onset of diarrhea and diagnosis by several years[32]. Pulmonary function tests showed a progressive decline in FEV_1 and vital capacity before diagnosis. Three of the four patients responded to antibiotic treatment and pulmonary function improved. A recent report described a patient with diarrhea, weight loss, arthralgia, progressive dyspnea, and a dry cough[33]. Small intestinal biopsy showed the typical changes of Whipple's disease. In addition, CT of the chest showed bilateral pleural effusion and thickening of interlobular septa, echocardiography showed pericardial effusion, and Doppler study showed severe pulmonary hypertension. Antibiotic treatment led to rapid symptomatic improvement and the pleural and pericardial effusion and the pulmonary hypertension resolved completely after three months of treatment. The authors assumed that pulmonary vascular involvement was the cause of pulmonary hypertension.

Central Nervous System

CNS involvement in Whipple's disease is recognized with increasing frequency and is the most devastating manifestation. Sieracki was the first to demonstrate PAS-positive macrophages perivascularly in the brain in two patients with Whipple's disease in 1959[31]. Since then, a number of case

reports have been published on patients who either presented with both gastrointestinal and CNS symptoms or in patients who presented with CNS relapse after successful treatment of gastrointestinal involvement[34-37].

CNS manifestations

- Dementia
- Ocular (Nystagmus,gaze paralysis)
- Cerebellar (Ataxia;myoclonus)
- Hypothalamic (Polydipsia;hyperphagia)
- Oculomasticatory myorhytmia

The presenting CNS symptoms include progressive dementia, ocular symptoms (nystagmus and vertical gaze paralysis), cerebellar symptoms (ataxia and myoclonus) and hypothalamic symptoms (polydipsia, hyperphagia, and insomnia). A recent review examined the presenting symptoms in 84 patients who presented with or developed CNS symptoms in the course of Whipple's disease published since 1966[38]. The diagnosis was made during life in 51 patients by tissue biopsy and at autopsy in the remaining 33 patients. Cognitive changes (71%), supranuclear gaze paralysis (51%), and altered level of consciousness (50%) were the most frequent CNS findings. A peculiar syndrome called oculomasticatory myorhythmia with constant horizontal eye movement (divergence and convergence) with simultaneous contractions of the masticatory muscles is considered pathognonomic for CNS Whipple's disease by some authors[39]. This syndrome was observed in 20% of the 84 patients. The final outcome of the 51 patients diagnosed during life was not analyzed in the review. There are a few case reports where the ocular manifestations have responded to antibiotic treatment but dementia has remained largely unresponsive[40-44].

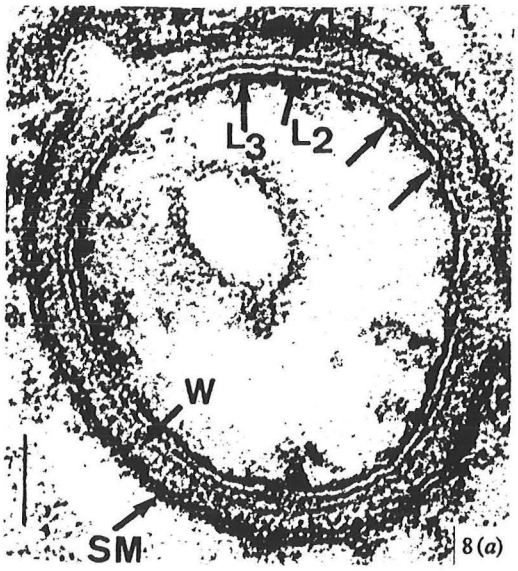
Eyes

Eye involvement in Whipple's disease is rare and may coexist with CNS involvement. The most common eye manifestations are uveitis, vitritis, retinitis, and retrobulbar neuritis. The diagnosis is often difficult as illustrated by a recent case report[45]. This patient, a 59 year-old woman, had seronegative arthritis for more than 10 years but had never had any gastrointestinal symptoms. She presented with bilateral uveitis. Whipple's disease was suspected early on but biopsies of duodenal mucosa failed to reveal PAS positive macrophages. She suffered progressive loss of vision over the ensuing four years when finally vitreous examination revealed PAS positive macrophages with rod-shaped bacilliform structures and EM revealed typical Whipple's bacteria. Her visual acuity improved with antibiotic treatment. A repeat duodenal biopsy at the time of diagnosis was again negative.

Sarcoidosis

Some patients with Whipple's may initially present with a sarcoidosis-like syndrome based on the presence of fever, arthralgia, adenopathy, and noncaseating granulomas in lymph nodes[46-49]. These patients were usually treated with prednisone and either failed to improve or developed frank gastrointestinal symptoms which then led to diagnostic biopsy. Some authors have suggested that antigenic material from degrading Whipple's bacteria, may elicit granuloma formation.

Identification of the Whipple's bacillus



The ultrastructure of the Whipple bacillus has been well characterized by EM by several groups of investigators[50,51]. It is a gram positive bacterium about 2μ long with a peculiar envelope consisting of three layers. The outer layer or surface membrane has a symmetric profile and has no PAS positive staining components. The cell wall is the middle layer and contains peptidoglycan. The inner layer faces the cytoplasm and has a triple layer profile. The unusual feature is the outer layer which is usually observed only in gram negative bacteria. The outer layer in Whipple's bacillus is thinner than that in gram negative bacteria and does not contain lipopolysaccharide based on staining characteristics. Normal appearing and dividing bacteria are observed extracellularly in the lamina propria whereas most of the bacteria phagocytosed by macrophages are in various stages of degradation with loss of the surface layer and cell wall. The bacterial remnants are surrounded by the innermost part of the cell wall composed of glycoproteins that react with the PAS stain and give rise to the brilliant magenta color characteristic of Whipple's disease. It should be emphasized that PAS positive macrophages are not pathognomonic of Whipple's disease but are

also observed in infections with mycobacterium avium intracellulare and histoplasmosis and in macroglobulinemia.

It was, however, still not possible to classify the Whipple's bacillus phylogenetically despite its morphologic characteristics because all attempts to culture the bacillus failed. It took a novel approach to finally classify the bacillus. Over the past decades, it has been realized that evolutionary relationships among organisms are more accurately determined by genotypes rather than phenotypic characteristics (culture conditions, biochemical characterization). A new specialty called molecular phylogeny has emerged in microbiology where classification of organisms is based on a molecular biologic approach[52,53]

Molecular phylogeny

- Classification of organisms by genotype
- Certain genetic sequences are conserved in all organisms
- Sequences acquire mutations at a slow rate
- Evolutionary distance among organisms is proportional to number of nucleotide differences between copies of same gene ('molecular clock')

There are certain genetic sequences that are conserved in all organisms where they encode essential biologic functions. All genetic sequences acquire mutations over time at a slow rate. It was therefore proposed that the evolutionary distance among organisms is proportional to the number of nucleotide differences among copies of the same gene and that certain genetic sequences could be used as 'molecular clocks[54].' The genetic sequence that has been most commonly used for phylogenetic analysis is the small subunit 16S ribosomal RNA (rRNA). Small subunit rRNA together with large subunit rRNA form ribosomes where proteins are synthesized. It is found in all cells and has a highly conserved structure. The linear structure of rRNA consists of regions of highly conserved sequences and regions with variable sequences. The entire sequence of rRNA from E. coli has been sequenced and is used as a numbering system for bacterial rRNA. Analysis of the variable sequences in different organisms allows the determination of phylogenetic and evolutionary relationships and forms the basis of new systems of natural classification. Sequence analysis of rRNA has been performed in a number of species and sequence comparisons have allowed the construction of a phylogenetic tree with three domains: Archaea, Eukarya, and Bacteria. There are large sequence differences in rRNA among the three domains.

Relman and coworkers were the first to use an rRNA-based approach to classify a previously uncultured bacterium from infected tissue of a patient with bacillary angiomatosis[55]. Bacillary angiomatosis is a vascular proliferative disease of the skin and lymph nodes seen mainly in immunocompromised patients. Warthin-Starry staining of the lesions shows clusters of bacilli but cultivation of the bacilli had been unsuccessful just as in Whipple's disease. The approach used was to extract DNA from infected splenic tissue and amplify bacterial rRNA with PCR using broad-range primers. These primers amplify only bacterial rRNA and not human rRNA. The amplified PCR product was cloned and sequenced. Disease-specific primers were designed from the variable regions of the sequence. The specific primers were used to amplify a PCR product from infected tissues of three additional patients with bacillary angiomatosis and in each case a band of the expected size was observed on agarose gel electrophoresis. The products were sequenced and were identical in sequence to that of the original patient. The sequenced PCR product was aligned with known bacterial rRNA sequences and was not identical to any known sequences. Optimized sequence alignment allowed the construction of a phylogenetic tree. The new sequence was similar to sequences observed within the alpha subdivision of purple eubacteria and most similar to Rochalimaea quintana, the agent that causes trench fever. The bacterium causing bacillary angiomatosis was named Rochalimaea henselae and later renamed Bartonella henselae. The successful identification of the bacterium and characterization of its phylogenetic relationship helped define the optimal culture conditions, and B. henselae can now be cultivated from infected tissue or blood[56].

Classification of Whipple bacillus

- Extract DNA from duodenal tissue from a patient with untreated Whipple's disease
- PCR with broad-range rRNA primers: 90% of rRNA sequence of Whipple bacillus
- PCR products cloned and sequenced-designed disease-specific primers
- PCR with specific primers:Identical product in original and 4 additional patients
- Phylogenetic analysis : Actinomycete

In 1992, Relman and coworkers used a similar approach to identify the Whipple bacillus[4]. DNA was extracted from a duodenal biopsy from a patient with untreated Whipple's disease and used for PCR reactions with broad-range bacterial rRNA primers. The PCR product was cloned and sequenced. The sequence of the product did not correspond to any known bacterial sequence. Two additional sets of broad-range primers were used to generate an almost full length (90%) sequence of the rRNA of the Whipple bacillus. A set of Whipple specific primers were designed from the sequence and used in

PCR reactions with DNA from four additional patients with Whipple's disease (formalin-fixed tissue in 3 and fresh-frozen tissue in 1). The reactions with the specific primers generated a product of the expected size in all five patients. The specific primers did not generate a product from several control tissues. Moreover, the specific primers failed to generate a product with DNA isolated from several bacterial cultures. Phylogenetic analysis of the rRNA sequence generated from this study suggested that the Whipple bacillus was an uncharacterized organism that belonged to a subdivision of gram positive bacteria with a high guanine and cytosine content characteristic of actinomycetes. A phylogenetic tree showed that the bacillus belonged to a subgroup of actinomycetes called actinobacteria. The authors suggested the name: Tropheryma whippleii gen.nov.sp.nov (trophe=nourishment and eryma=barrier). Most of the actinomycetes are found in the soil or water. Other investigators have found an rRNA sequence identical to that reported by Relman and also confirmed the taxonomic placement of the Whipple bacillus as an actinomycete[57]. The Whipple specific primers have now been used by several investigators in PCR based diagnoses of Whipple's disease. (vide infra). Subsequently, an rRNA sequence-based approach has been used to identify the agents that cause human ehrlichiosis and hantavirus pulmonary syndrome[58].

The design of Whipple specific primers for PCR analysis led investigators to start a search for the natural occurrence of the bacillus in the environment. Since soil and water bacteria tend to concentrate in sewage the search was initiated with samples from five sewage treatment facilities in Germany[59]. A total of 38 waste water samples were analyzed with PCR and 25 samples generated a product of the expected size. Nine of these samples were cloned and sequenced and in each case the sequence was identical to the known sequence of the Whipple bacillus. It is thus possible that the Whipple bacillus is a regular member of the polymicrobial communities that exist in sewage since a positive product was found in all five plants. The exact way a person gets exposed is still unknown but oral ingestion has been suspected because intestinal involvement is a typical feature. It is also interesting to note that many patients with Whipple's disease have an outdoor profession.

Immunologic defect in patients with Whipple's disease?

The predilection of Whipple's disease to mainly strike middle-aged, white men has led to the suggestion that patients who develop the disease have an underlying immune defect.

- Cutaneous anergy -common finding
- Macrophage dysfunction :

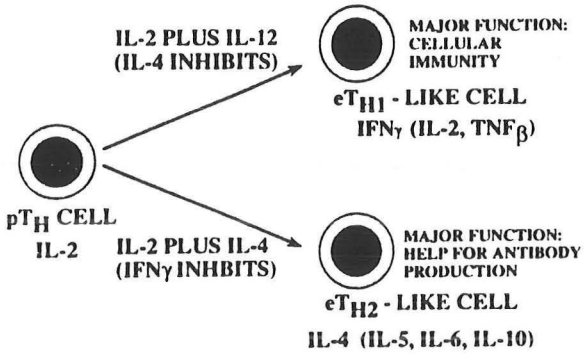
  Defective bacterial degradation

  Decreased expression of CD 11b

  Decreased production of interleukin 12

Cutaneous anergy to a variety of antigens and decreased lymphocyte response to mitogens have been observed by several investigators[60,61]. The anergy persists in patients who have concluded a successful treatment and have remained asymptomatic which suggests a defect in cell-mediated immunity. In a study of isolated monocytes and macrophages from a Whipple's patient, it was observed that phagocytosis of pathogens was normal but intracellular degradation was impaired[62,63]. This patient was studied both during relapse and in remission and macrophage dysfunction persisted. Further evidence for possible macrophage dysfunction was obtained in a large study of Whipple's patients (n=27) with either active disease or in remission where all patients had a persistent reduction in circulating mononuclear cells expressing complement receptor 3α-chain (CD11b)[64]. It was postulated that the reduction of this cell population may lead to inadequate macrophage activation and bacterial degradation as this receptor is involved in phagocytosis. It has also been shown by immunohistological studies that PAS-positive macrophages in the lamina propria in Whipple's disease do not express CD11b[65]. Cytokine production in monocytes and macrophages isolated from peripheral blood in patients with Whipple's disease has recently been studied[66]. Interleukin 12 (IL-12) production in stimulated monocytes was significantly reduced both in patients with active or inactive disease whereas production of TNFα, IL-10, and TGBß were similar to controls. It was also found that IFN-γ production by T cells was reduced in these patients. The authors concluded that patients with Whipple's disease have a primary defect in monocyte IL-12 production that in turn leads to reduced T cell production of IFN-γ. IL-12 is produced by monocytes and macrophages in response to phagocytosis of infectious agents.

IL-4 AND IL-12 MUTUALLY REGULATE CHOICE OF CYTOKINE-PRODUCING PHENOTYPE



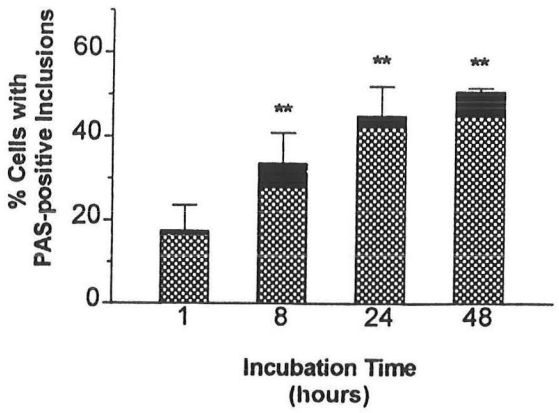
It is now commonly accepted that naïve T_H cells can be induced to elicit either a T_{H1} or T_{H2} response characterized by their unique cytokine production (T_{H1}:IL-2; IFN-γ and TNFβ; T_{H2}:IL-4; IL-5; IL-6;IL-10; and IL-13)[67-69]. A T_{H1} response develops during infection with intracellular bacteria, protozoa, and viruses whereas a T_{H2} response predominates during extracellular helminthic infections. For example, Leishmania major, a protozoa, causes an

intracellular infection of macrophages which leads to a vigorous T_{H1} response with activation of macrophages by IFN-γ and killing of intracellular L. major in most strains of mice, and the mice survive[70]. T_H cells isolated from these mice produce IFN-γ upon activation with L. major antigens. However, in BALB/C mice, L. major causes an overwhelming infection and death. Isolated T_H cells from these mice produce IL-4 and the other cytokines characteristic of a T_{H2} response. Leprosy caused by the still uncultured mycobacterium leprae is a human example of either a T_{H1} or T_{H2} response. Lepromatous leprosy is characterized by a striking accumulation of bacteria in macrophages and IL-4 production in stimulated T_H cells (T_{H2} response), whereas there are very few organisms in macrophages in tuberculoid leprosy and stimulated T_H cells isolated from these patients produce IFN-γ (T_{H1} response)[71]. These observations in mice and man suggest that the selection of a T_H cell response is determined in part by genetic factors.

The inflammation in lamina propria of the small intestine in patients with Whipple's disease is characterized by accumulation of bacteria within macrophages and a paucity of inflammatory cells that suggests a T_{H2} response. A proof of this hypothesis would require isolation of monocytes from Whipple's patients and stimulation with antigenic material from the Whipple bacillus with demonstration of IL-4 production. The impairment in IL-12 production observed in Whipple's patients might be the primary defect that results in a presumed T_{H2} response when a T_{H1} response would be expected due to an intracellular infection. Whether this defect is the result of an inherited disorder of macrophage function or is due to certain characteristics of the Whipple bacillus is still unknown. It should be noted that patients who eventually develop Whipple's disease have not shown an increased susceptibility to other infections earlier in life. Defective IL-12 production has also been observed in a rare familial, chronic relapsing form of mycobacterium avium infection[72]. This rare disease has been successfully treated with recombinant IFN-γ which suggests that IFN-γ may also be used in the treatment of Whipple's disease[73].

Cultivation of the Whipple bacillus

Cultivation of the Whipple bacillus has frustrated numerous investigators. A variety of culture conditions have been used with infected intestinal tissue and a number of pathogens have been isolated but never in a reproducible manner which serves to prove that this actinomycete has unique culture requirements[74]. A group of Swiss investigators have recently used a novel approach to cultivate Listeria in macrophages which had been deactivated by IL-4[75]. IL-4 has no effect on the phagocytic activity of macrophages but it downregulates microbicidal activity by a yet unknown mechanism allowing Listeria to multiply within macrophages. The same group has now used a similar approach to successfully cultivate the Whipple bacillus in isolated human monocytes[5].



Time-dependent growth of Whipple bacilli

The starting material was infected aortic valves from two patients undergoing aortic valve replacement for aortic insufficiency. Whipple's disease was not suspected at the time of surgery in either patient but was proven by PAS staining, EM and PCR analysis. Homogenized heart valve tissue was added to monocyte cultures inactivated by IL-4. The cultures were examined after 8 – 10 days incubation and monocytes were found to be filled with PAS-positive rods. EM showed intact and degenerating bacteria at this time point, and the identify of the Whipple bacillus was confirmed by PCR. They were able to propagate the bacillus in large volume cultures and show time-dependent growth indicating multiplication in monocytes. It is of interest that extracellular growth was never observed which is at variance with observations on human tissue. The ability to propagate the Whipple bacillus in *in vitro* culture should allow the definition of optimal growth conditions and biochemical characterization. It may also allow the establishment of a serologic diagnostic test and drug susceptibility testing[76]. The results of this study, if confirmed by other investigators, is a major breakthrough in the pathogenesis of this rare disease.

Diagnosis

The diagnosis of suspected Whipple's disease is confirmed by histologic examination of affected tissues. The finding of PAS-positive macrophages is almost pathognomonic. The examination should include an acid-fast stain to exclude M. avium infection. Histoplasmosis is excluded by the characteristic features of the fungus. When available, EM can be used to demonstrate the characteristic bacillus and EM has, until recently, been considered the definite proof of the diagnosis. In fact, Dobbins, a life-long student of Whipple's disease, considers the morphologic features of the bacillus to be unique. The introduction of a PCR method for diagnosis may now supplant EM. The utility of PCR diagnosis has been evaluated in two large retrospective studies of 35 and 30 patients, respectively, with histologically confirmed Whipple's disease[77,78].

Diagnosis

- Demonstration of PAS pos macrophages in affected tissue (r/o MAI and histoplasmosis)
- Demonstration of bacillary bodies by EM
- PCR with Whipple-specific primers

In the first study, the specificity was tested with 37 control bacterial strains and with intestinal biopsies from 16 control patients and the PCR reaction was consistently negative in all samples. The sensitivity was tested in intestinal biopsy samples from 35 Whipple patients. Thirty samples were formalin-fixed and generated a PCR product of expected length, whereas 5 samples were Bouin-fixed and negative in the PCR reaction. Follow-up biopsies during and after treatment were obtained in 24 patients and 23 converted to a negative reaction within one year. However, three of those with negative PCR reactions with intestinal tissue later developed CNS Whipple's disease. In the study of 30 patients with Whipple's disease from the Mayo Clinic, 29 had a positive result on PCR reaction (sensitivity 96.6%). An additional 8 patients had intestinal histology suggestive of Whipple's disease and 7 of these had a positive PCR reaction. Follow-up intestinal biopsies were obtained in 17 patients after treatment. Five patients were in remission and had normal histology and negative PCR but the remaining 12 patients had a positive PCR result. Seven of the 12 patients were in relapse or had not responded to treatment. It is of interest that 8 of the 12 patients with positive PCR had normal intestinal histology which serves to emphasize the extreme sensitivity of PCR. CNS involvement in Whipple's disease is usually diagnosed by neurologic symptoms or sometimes by demonstrating a mass lesion by CT or MRI, but rarely documented by brain biopsy. Analysis of CSF may reveal elevated protein concentration and sometimes PAS-positive macrophages. In a recent study of CSF by PCR in 18 patients with histologically confirmed intestinal Whipple's disease, but without CNS symptoms, 6 patients were found to have a positive PCR reaction at the time of diagnosis[79]. The question whether PCR analysis should supplant the demonstration of PAS-positive macrophages as the gold standard for the diagnosis is still debatable and will require a prospective study[80]. Perhaps the greatest utility of PCR analysis will be in the diagnosis of atypical presentation as illustrated by a recent report of five patients with initial diagnoses of immune thrombocytopenia, juvenile rheumatoid arthritis, lymphadenopathy, myopathy, and quadriparesis, respectively[81]. All five patients had normal jejunal histology, but PCR was positive for Whipple's disease with intestinal and other tissues in all five patients, and their symptoms improved with antibiotic treatment.

Treatment

Treatment of Whipple's disease still rests on empirical observations. A variety of antibiotics have been employed since Paulley's first report on the successful use of chloramphenicol in 1952. All the reports on the effect of antibiotics are retrospective studies. The first small series of patients was reported from Duke University in 1966 where 10 patients were treated with a short course (1 to 3 weeks) of antibiotics (penicillin, streptomycin or tetracycline)[82]. All patients went into remission but 7 patients relapsed after months to years. A second course with the same antibiotics but of longer duration induced a remission in six of the seven patients. The authors recommended an initial treatment with penicillin (1.2 mil U per day), and streptomycin (1 gram per day) for 2 weeks followed by tetracycline 1 gram daily for 3 to 6 months. This regimen became the preferred treatment of Whipple's disease over the following decades until newer studies questioned the efficacy of tetracycline.

Relapse after antibiotic treatment

Antibiotic	# of relapses			Total	
	Ref 83	15	16		
Tetracycline	21/49	2/16	5/28	28/93	30%
Penicillin+strepto	4/20	0/7	0/7	4/34	12%
TMP-SMX	0/3	0/0	0/12	0/15	--
Others	6/16	0/2	2/11	8/29	28%
			Total	40/171	23%

Dobbins and coworkers conducted a large retrospective study of 88 patients collected from a number of institutions in the U.S., Canada, and Europe on the outcome of antibiotic treatment[83]. All patients had been followed for at least one year after completion of treatment or two years since initial diagnosis. Fifty-seven patients did not relapse but 31 patients suffered a relapse. The mean time to relapse was 4.2 years. The most common type of relapses were clinical (16) and CNS symptoms (13). The most important observation from this study was that most relapses occurred in patients who had been treated with tetracycline alone (21 relapses in 49 patients). CNS relapses responded poorly to a second course of antibiotics, only one of eleven in whom follow-up was available responded, whereas all the non-CNS relapses responded to a variety of antibiotics. It was concluded that tetracycline is not adequate therapy for Whipple's disease because it does not cross the blood/brain barrier. Only three patients were treated with trimethoprim-sulfamethoxasole (TMP-SMX) alone but none suffered a relapse. The authors recommended an initial 2 week treatment with parenteral penicillin (1.2 mil U) and streptomycin (1 gram) followed by TMP-SMX DS (1 tab b.i.d.) for one year. A recent study from Germany compared

the efficacy of tetracycline and TMP-SMX in inducing clinical remission in 30 patients with Whipple's disease[84]. Twenty-two patients were treated with tetracycline and eight patients with TMP-SMX. Five patients were switched from tetracycline to TMP-SMX because of lack of response. Trimethoprim-sulfamethoxasole induced a complete clinical remission in 12 of 13 treatments and tetracycline only in 13 of 22 treatments. The difference in treatment outcome was statistically significant and supports the treatment recommendations of Dobbins' study. The one treatment failure with TMP-SMX in this study occurred in a patient with advanced CNS disease at the time of diagnosis. In addition, one patient who had a complete clinical remission with TMP-SMX developed CNS symptoms after 14 months of continuous treatment. In a recent report on CNS Whipple's disease, 3 of 5 patients treated with TMP-SMX developed CNS relapse during continuous treatment while 4 patients treated with third generation cephalosporins did not relapse[85]. TMP-SMX crosses the blood/brain barrier and achieves high intracellular concentrations but it is only bacteriostatic. These authors therefore suggested that the initial treatment of Whipple's disease should be i.v. ceftriaxone (2 grams b.i.d.) and streptomycin (1 gram q.d.) for 2 weeks, followed by oral TMP-SMX (960 mg b.i.d.) or oral cefixime (400 mg q.d.) for one year[86]. As is evident, these retrospective studies are far from perfect and treatment recommendations are based on the outcome in a small number of patients. It is, however, possible that the optimal culture conditions for the Whipple bacillus will be defined in the near future and, thus, facilitate susceptibility testing. Only then will the choice of antibiotics rest on solid evidence and, hopefully, result in cures without relapses.

Conclusions

There has been slow but steady progress in our knowledge of Whipple's disease since its original description more than ninety years ago. It remains a rare disease that mainly afflicts middle-aged, Caucasian males. The reason for its rarity and its predilection for a certain subset of the population remains unclear. Current hypotheses center around infrequent exposure to the bacillus, still undefined characteristics of the bacillus, or inherent macrophage dysfunction. The evidence that Whipple's disease is a bacterial infection is now generally accepted although all of Koch's postulates have not been fulfilled. The diagnosis is still difficult especially in atypical presentation. The introduction of a PCR-based diagnostic test will undoubtedly be helpful as a general diagnostic tool and even more in questionable cases. It is a curable disease when treated with appropriate antibiotics but the choice of antibiotics and length of treatment still rest on empiric grounds.

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