Tropheryma whipplei infection and Whipple's disease

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Recent advances in medical microbiology, epidemiology, cellular biology, and the availability of an expanded set of diagnostic methods such as histopathology, immunohistochemistry, PCR, and bacterial culture have improved our understanding of the clinical range and natural course of *Tropheryma whipplei* infection and Whipple's disease. Interdisciplinary and transnational research activities have contributed to the clarification of the pathogenesis of the disorder and have enabled controlled trials of different treatment strategies. We summarise the current knowledge and new findings relating to *T whipplei* infection and Whipple's disease.

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Introduction

Whipple's disease is a rare, systemic illness often characterised by weight loss, diarrhoea, and arthralgia. George H Whipple suspected an infectious disease at his first description of the disorder in 1907.[1] Further findings suggested a bacterial cause: macrophages with cytoplasmic periodic acid-Schiff (PAS) inclusions were described in 1949,[2] and bacteria were observed by electron microscopy in 1960.[3–5] The disease was fatal until the first successful antibiotic treatment was introduced in 1952.[6] The causative organism was finally identified in the 1990s by sequencing of its 16S ribosomal RNA genes.[7,8] The first cultivation of the bacterium in macrophages was described in 1997,[9] and, in 2000, stable cultures were established[10] enabling sequencing of *Tropheryma whipplei's* whole genome 3 years later.[11,12] The aim of this Review is to summarise the pathogenesis, diagnosis, and treatment of Whipple's disease. For example, immunosuppressive treatment (under the misdiagnosis of a rheumatic disorder) was recognised to influence the course of Whipple's disease. In addition, we aim to enhance the awareness of life threatening complications during treatment like immune reconstitution inflammatory syndrome (IRIS) that occurs particularly in patients following immunosupressive treatment.

Microbiology of *T whipplei*

T whipplei, the causative bacterium of Whipple's disease, is a rod-shaped organism that can be visualised by electron microscopy (figure 1).[2–4] The bacterium has a trilaminar plasma membrane that is surrounded by a homogeneous cell wall.[1–4] Genomic amplification by PCR was used to detect bacterial 16S RNA gene from duodenal lesions of patients with Whipple's disease.[7,8] The analysis enabled the phylogenetic characterisation of the new genus *Tropheryma*.[8]

The organism was first propagated, at least for a short time, from heart valve tissue in peripheral blood mononuclear cells that had been immunologically deactivated.[9] Stable cultures were established by Raoult and co-workers in human fibroblasts using heart valve tissue from a patient with endocarditis.[10,13] *T whipplei* has a very long doubling time (up to 18 days); however, it can be shorter in special mammalian cell-free (axenic) growth media.[10,14] Cultivation of the bacillus from sterile fluids is easier, because contaminated specimens require the use of antimicrobial drugs. So far, a large number of strains have been obtained from cardiac valves, blood, synovial fluid, cerebrospinal fluid, duodenal biopsies, stool, saliva, and bronchoalveolar fluid.[10,15–17] The digestive lumen is the probable site of multiplication of *T whipplei*, where it is taken up by phagocytosis and ineffectively degraded in macrophages. *T whipplei* replicates within mucosal macrophages and peripheral blood mononuclear cells.[16]

The genome of two different strains (TW08/27 and Twist Marseille) of *T whipplei* contains 925 kilobase pairs, 800 protein-coding genes, and has a guanine-cytosine content of 47%,[11,12] characteristics that are common in intracellular bacteria. Phylogenetic and 16S–23S rRNA intergenic sequence analysis determined the organism as Actinobacteria, taxonomically located between the subdivision of Gram-positive Actinomycetes and the Cellulomonadaceae.[8,18]

T whipplei is deficient in genes that encode energy metabolism and aminoacid synthesis (eg, absence or impairment of 16 aminoacids, no thioredoxin, and no thioredoxin reductase homologues). Genome sequence alignment showed chromosomal inversions and the presence of highly conserved common repeats, which argues for a high genetic diversity and the capability to express many cell-surface proteins. Thus, the frequent genomic rearrangements might hint at an adaptive response to the host defence and environmental conditions.[11,12]

Several in-vitro studies show that many French and German strains of *T whipplei* are naturally (genetically) resistant to trimethoprim, and also frequently to sulfadiazine.[19–21] By contrast, doxycycline is an effective bactericidal treatment for *T whipplei* infection in vitro, particularly if it is combined with hydroxychloroquine (which acidifies the phagolysosomes).[19–22]

Natural habitat, prevalence, and transmission of *T whipplei*

Exposure to contaminated soil (eg, by farmers) has been postulated to be a possible route of infection.[1] Actinobacteria are environmental microorganisms found in soil, freshwater, or seawater sediments; therefore, it is not surprising that *T whipplei* has been found in 37–66% of influxes to sewage plants.[15,23–25]

T whipplei is frequently detected in stool and saliva samples from patients with Whipple's disease and, to a small degree, in asymptomatic carriers (roughly

2–4%).[16,24,26] *T whipplei* has been detected in stool samples in 1–11% of healthy individuals,[26] and in 12–26% of sewage plant workers.[24] The organism has been detected in saliva samples in 0·2% of healthy individuals,[26,27] and in 2·2% of sewage plant workers.[27] Carriage of *T whipplei* in healthy individuals depends on the geographical area—eg, prevalence is much higher in Senegal (31% in stool samples and 3·5% in saliva)[28] than in Europe.[24–27] Genomic variants of the bacterium are associated with neither the geographical residence of the patients nor the organotropism of the agent.[29] Other factors associated with the risk of *T whipplei* infection or colonisation are close contact with carriers of the organism or patients with Whipple's disease within families (infection with the same genotypes);[30] poor living conditions of homeless people in shelters;[31] and absence of toilets.[32] Acute and self-limiting *T whipplei* infection occurs via the respiratory route (*T whipplei* causes pneumonia in children and is detected in bronchoalveolar lavage samples),[17,33] and via the gastrointestinal route (identical *T whipplei* genotypes have been identified in children during episodes of *T whipplei* gastroenteritis).[33] Asian children from Laos frequently (51 [48%] of 106 children studied) carry *T whipplei* in their stools.[34] The bacterium is viable in human faeces and saliva.[27]

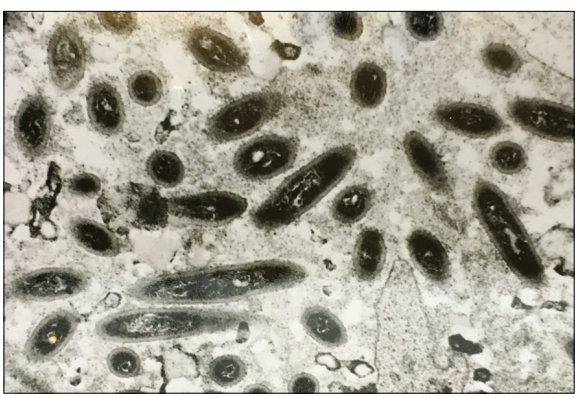

Figure 1: **Morphology of *Tropheryma whipplei* as visualised by electron microscopy**
Electron microscopic view of *T whipplei*. The characteristic, rod-shaped (0·25 × 2 μm) organism is found typically in macrophages of the lamina propria of the small intestine. It can be observed in the extracellular space in florid disease or within cells in various stages of degradation. *T whipplei* shows a trilaminar plasma membrane, a surrounding homogeneous cell wall, and an outer trilaminar membrane-like structure (magnification ×20 000).

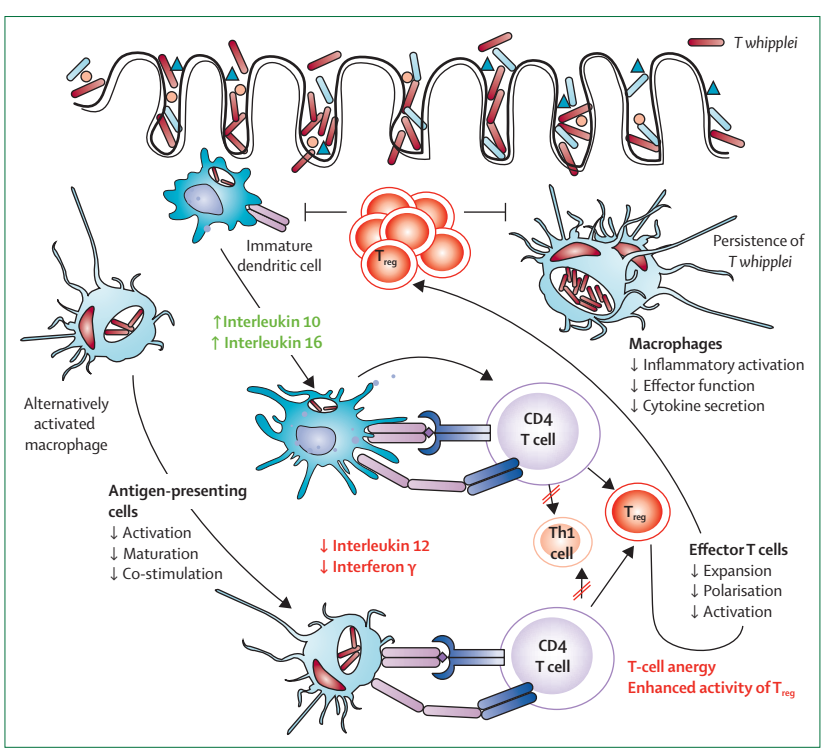

Figure 2: **Pathogenesis of Whipple's disease**
Model for the pathogenesis of Whipple's disease: an inappropriate maturation of antigen-presenting cells caused by the presence of interleukin 10 and interleukin 16, and the absence of interferon γ and interleukin 12, might lead to insufficient antigen presentation and inhibit the stimulation of antigen-specific T-helper-1 (Th1) cells while stimulating the proliferation of regulatory T cells (T_{reg}). Subsequently, macrophages within the affected tissues are alternatively activated, enabling the persistence of *Tropheryma whipplei*.

All these findings support human-to-human transmission of *T whipplei*. Environmental studies of the possible sources of *T whipplei* (eg, dust, specimens from domestic animals, ixodid ticks, and fleas) were PCR negative.[23,31,32] Because human beings are the only known host of *T whipplei*, and all environmental sources are in connection with human waste, the agent might have spore-like formations facilitating survival in the environment, thus explaining its high tenacity, even against glutaraldehyde. Although spores have not yet been identified, *T whipplei* possesses regulatory factors essential for sporulation.[12]

Pathogenesis of Whipple's disease

Chronic systemic infection with *T whipplei* seems to occur only in predisposed patients who show a lifetime susceptibility to *T whipplei* infection;[35] on the one hand Whipple's disease is very rare and usually develops over the course of many years or even decades (insidious replication), while on the other hand asymptomatic carriage of *T whipplei* is frequent.[26–28,36–38] A specific genetic predisposition involves HLA associations (HLA alleles *DRB1*13* and *DQB1*06*) that interfere with the optimum presentation of antigens,[39] *IL16* gene polymorphisms,[40] and other polymorphisms that polarise cytokine production towards T-helper-2 (Th2)-cell activity.[39,41] This specific genetic predisposition probably explains the lifetime susceptibility of patients with Whipple's disease and, from the clinical point of view, the repeated and very late relapses in some individuals.[1,15,35] Beyond the genetic basis, immunosuppresion has a pathogenetic role. HIV infection has been reported to enhance the risk of carriage of *T whipplei*.[37,42,43] Erroneous medical immunosuppression of patients with Whipple's disease can accelerate gastrointestinal symptoms[44–46] and complicate the course of the disease.[44,46]

Upon contact with *T whipplei*, most infected individuals subsequently develop a protective immune response.[38,47,48]