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Editorial: The Whipple Bacillus Lives (Ex Vivo)!

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Cultivation of the bacillus associated with Whipple's disease, *Tropheryma whippelii,* has been an elusive goal for many generations of clinicians and microbiologists familiar with this disease. The desire to identify this enigmatic organism has motivated many of these efforts. Many purported successes have later proven erroneous, and many more unsuccessful attempts have never been reported [1]. Cell-free media, animal cells, and animals themselves have all been used, resulting in recovery of a wide range of bacterial species, including members of the *Corynebacterium, Streptococcus, Propionibacterium,* and *Haemophilus* genera. The rough resemblance of *Rhodococcus equi*–, *Mycobacterium paratuberculosis*–, and *Mycobacterium avium* complex–associated diseases in foals, cows, and humans, respectively, to Whipple's disease has been noted; however, pathology closely mimicking that of the latter has never been knowingly and intentionally transferred to another human nor reproduced in a nonhuman host. Despite the unusual cell wall features of this bacillus and its reactivity to the periodic acid–Schiff (PAS) reagent [2], the absence of a specific microbial signature has greatly hindered efforts to evaluate these previous cultivation efforts.

Over the past 10 years, there has been a fundamental change in the approach to microbial identification and taxonomy. This change involves a decreasing reliance on cultivated organisms and their associated phenotypes, such as morphology, antigenicity, and biochemical activities, and an increasing reliance on genotype, that is, nucleic acid sequences [3, 4]. Certain genes, such as that of the small subunit ribosomal RNA (ssu rDNA), accurately reflect the evolutionary history of the entire genome and allow one to determine the relationships of any given organism with all others. By taking advantage of interspersed, highly conserved portions of these genes, one can amplify the intervening, phylogenetically useful sequence directly from infected clinical specimens and identify a previously uncharacterized or novel microbial pathogen [5, 6]. With this "broad-range polymerase chain reaction" (PCR) method, a unique, previously unrecognized bacterial ssu rDNA sequence was amplified from multiple independent Whipple's disease tissues [7, 8]. Phylogenetic analysis of this sequence suggested that the Whipple bacillus is an actinomycete and prompted the proposal of a new taxon, *Tropheryma whippelii* [8]. The *T. whippelii* ssu rDNA sequence now provides the basis for a specific PCR detection assay [8–11]. Armed with this diagnostic tool, Schoedon et al. [12] have tested a clever approach for in vitro Whipple bacillus propagation. The outcome of host infection depends in part upon a complex, local interplay of immune effector cells and cytokines. Pathogens often manipulate these host immune responses to render the local environment more hospitable and to enhance their survival or dissemination [13]; one strategy is to alter the local Th1/Th2 helper T cell profile. Suppression of tumor necrosis factor-α or interferon (IFN)-γ–mediated macrophage activation is a common strategy for microorganisms that choose an intracellular niche. Might one mimic this strategy by treating macrophages with cytokines or hormones that deactivate microbicidal pathways but preserve phagocytosis and thereby promote replication of an organism in a protected intracellular compartment? Interleukin (IL)-4, IL-10, and dexamethasone have been shown to enhance intracellular growth of certain pathogens within human macrophages by suppressing both oxidative and nonoxidative killing mechanisms but without inhibiting bacterial uptake [14, 15]. Schoedon et al. have taken this same approach for cultivating the Whipple bacillus. In this issue of the *Journal,* they provide evidence that *T. whippelii* replicates in the laboratory within human peripheral monocyte–derived macrophages, as well as within a macrophage-like cell line, when these host cells are treated with IL-4 [12].

The potential ramifications of these findings by Schoedon et al. are extensive. Yet, given the long and frustrating history of this disease and organism, one must evaluate this report carefully. In the absence of direct bacterial quantification and any obvious extracellular growth in vitro, how strong is the evidence for microbial replication? The authors relied on two types of data: (1) an increase in both the percentage of cells with visible PAS-positive inclusions and in the number of inclusions per cell and (2) PCR-based detection of *T. whippelii* DNA sequences after a number of cell passages sufficient to eliminate DNA detection after an equivalent inoculum dilution alone, that is, 100- to 1000-fold (in the absence of host cells). In theory, the first type of data might be explained in part by more rapid death of uninfected host cells (leading to an increase in the percentage of PAS-positive cells), although this seems unlikely, and by intracellular redistribution and trafficking of PAS-positive bacterial cell wall. PAS reactivity is difficult to quantitate and is only an indirect marker of bacterial number.

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However, quantitative PCR methods with internal standards would provide a more direct and reliable assessment of bacterial growth in this situation [16].

The exact identity of the organism propagated in this study is another crucial issue. Heart-valve tissue with apparently typical Whipple's disease pathology, from 2 persons, served as the inocula for cultivation [12]. From each tissue sample, a partial bacterial 16S rDNA fragment was amplified using broad-range PCR, from which ~400 bp of sequence was determined. Although these sequences were nearly identical to the corresponding segment of the previously published *T. whippelii* 16S rDNA sequence [8], this amount of primary sequence information is less than desirable. At various stages of tissue cocultivation, a *T. whippelii* PCR-based assay was positive, but the details of assay specificity are not provided. For the purpose of species, and certainly strain, identification, a complete 16S rDNA sequence is probably a minimum requirement; in most cases, additional sequence information from more rapidly evolving genetic loci is necessary. Nonetheless, it appears quite likely that the organism propagated by Schoedon et al. is either *T. whippelii* or a close relative.

What have we learned about the Whipple bacillus and its host from the results of this study? Might we have anticipated these findings from information previously available concerning this organism and its associated disease? First of all, *T. whippelii* appears to have a particular affinity for human macrophages and macrophage-like cells. Perhaps it is no coincidence that this organism elicits a prominent macrophage response during natural infection. Whether or not *T. whippelii* warrants the designation as an "intracellular pathogen," however, is unclear. The cell cocultivation conditions chosen by Schoedon et al. may have biased the outcome toward intracellular survival and growth and may not have provided the bacterium with the extracellular conditions that it encounters and prefers in a susceptible host. The pathology of Whipple's disease is notable for numerous intact extracellular bacilli, with some undergoing binary fission; at the same time, most bacilli within macrophages are at least partially degraded [1]. The same observation was made by Schoedon et al. in vitro, despite the macrophage-inactivating effects of IL-4 [12]. Second, the Whipple bacillus is at least microaerophilic. Third, the requirement for IL-4 treatment of macrophages draws attention to the possibility of a host cellular immune defect and the potential role of a polarized Th2 cytokine profile. Bjerknes and colleagues [17, 18] have suggested that monocytes and macrophages from Whipple's disease patients exhibit deficient microbial degradation capabilities. On the other hand, the effects of IL-4 are pleiotropic and nonspecific; the link between a Whipple's disease host defect and IL-4 may be only indirect. In theory, IFN-γ and its receptor are also possible key players in host susceptibility to this disease. Finally, did phylogeny predict physiology? When the relevant branch of the evolutionary tree is robust, microbial phylogeny sometimes predicts preferred growth conditions; however, because there are few known close relatives of *T. whippelii,* such insights would have been difficult to discern.

In an era of decreasing reliance on cultivated organisms and increasing reliance on rapid and specific molecular or sequence-based methods for microbial characterization, what is the value of propagating an organism such as the Whipple bacillus in the laboratory? With phylogenetically useful sequence alone, microbial identification and evolutionary analysis are possible; predictions can be made regarding metabolic, biochemical, and virulence-associated activities and then further evaluated with consensus PCR and sequencing; growth state might be estimated from quantitative rRNA measurements; and compelling arguments can be developed for a role in disease causation [19]. To the degree that additional genome sequence information may be further revealing, one might "walk" the chromosome of an uncultivated microorganism beginning with the ssu rDNA [20]; it may even be possible to determine a complete genome sequence from such organisms with shotgun cloning methods and powerful sequence assembly algorithms. However, the advantages of a laboratory propagated organism are still substantial.

A viable microorganism, provided with relevant growth conditions, readily reveals its metabolic and virulence capabilities. Disease models and correlates of pathogenicity can be established. Laboratory propagation creates substantial amounts of pure microbial cell mass, with which serologic assays can be developed, monoclonal antibodies elicited, and chromosomal DNA easily prepared. From recombinant chromosomal libraries, virulence-associated genes can be isolated, and the molecular mechanisms of disease causation can be explored. Immunodominant antigens can be cloned and expressed. Diagnosis can then be based on whole cell– or recombinant antigen–based serologic assays or on specific immunochemical and immunofluorescent tissue hybridization. Microbial drug susceptibility can be assessed in vitro. Recombinant antigens may be protective for susceptible hosts.

In theory, all of these advantages can now be realized for *T. whippelii.* In practice, several issues will first need to be addressed. The organism propagated by these authors should be characterized in greater detail. Optimization of growth conditions leading to consistent, high-titer culture yields will be important. One approach might involve cell lines bearing transgenes or genetic defects that render them hypersusceptible to *T. whippelii* growth. Intracellular bacterial degradation needs to be minimized. And, of course, the findings reported herein need to be reproduced by others. But if this work is substantiated, Schoedon and colleagues will have made a key contribution to a fascinating 90-year saga in clinical microbiology. No microorganism is uncultivatible; the real issue is whether we are intelligent enough to understand the sometimes complex and intimate growth requirements of our prokaryotic cousins.

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