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COMMENTARY

Cultivation of Whipple bacillus: the irony and the ecstasy

For 90 years investigators (including George Whipple) have been unable to propagate the microbe responsible for Whipple's disease, despite many published and probably many more unpublished attempts.[1] Evidence that a bacterium causes Whipple's disease has accumulated in recent decades. Light and electron microscopy show rod-shaped bacilli in the affected tissues of these patients, usually free in the lamina propria of the small bowel but also as partly degraded structures within macrophage vacuoles.[2] On the basis of histology, investigators have suggested that these organisms propagate extracellularly,[3] although intact organisms have been detected within several types of cells.[4] A molecular phylogenetic approach provides evidence that the Whipple bacillus is a member of the actinomycete taxon of bacteria.[5,6] DNA from this microbe, unofficially named *Tropheryma whippelii*, is almost invariably present in patients who have Whipple's disease, but disappears with effective antibiotic treatment.[7] Using both histological and molecular data, G Schoedon and colleagues[8] now provide evidence of the successful propagation of *T whippelii* in cell culture. Interleukin-4 (IL-4) was critical for rendering peripheral blood monocytes and monoblastic cell-lines permissive for intracellular growth of the organism.

It is ironic that the very cell believed to destroy the Whipple bacillus has been used to nurture its replication in the laboratory. This contradiction raises several new questions about the bacillus and how it interacts with the host. First, propagation of *T whippelii* within the artificial environment created in the laboratory may have little relevance to what occurs in natural infection. However, successful laboratory cultivation of *T whippelii* in macrophages suggests that some bacilli may survive and replicate in vivo within the macrophages of patients. If true, then the microbe may spread to other tissues as an intracellular passenger, and the macrophage may serve as a reservoir of infection, protecting *T whippelii* from immune defences. Whipple's disease is notable for a disturbingly high rate of relapse despite months to years of antibiotic treatment. Can the microbe persist within macrophages of some patients, leading to subsequent reactivation disease, in a manner analogous to tuberculosis? On a more practical level, the in-vitro propagation of *T whippelii* paves the way for much needed antibiotic susceptibility testing, preparation of purified antigens for serological testing, and further characterisation of this enigmatic microorganism.

Why is the laboratory propagation of *T whippelii* in macrophages dependent on IL-4, and does this requirement have clinical relevance? IL-4 promotes differentiation of T cells towards a TH2 (helper) phenotype, which leads to B-cell activation and macrophage downregulation.[9] By contrast, IL-2 promotes differentiation towards a TH1 (inflammatory) phenotype, activating macrophages with γ-interferon and tumour-necrosis factor. Is successful human infection with *T whippelii* dependent on secretion of IL-4 and induction of a macrophage-deactivating TH2 response? If macrophages are deactivated, why do they seem to be degrading bacilli under electronmicroscopy? If a TH2 response is needed for infection, is this response determined by host genetic predisposition, or is it orchestrated by the bacterium? Although an immune defect has been postulated in patients with Whipple's disease, no specific defect has been described. The time is now ripe for investigating abnormalities in cytokine activity and T-cell differentiation in these patients. Furthermore, the therapeutic use of macrophage-activating cytokines, such as γ-interferon and tumour-necrosis factor, should undergo further testing. The possibility remains, however, that patients with Whipple's disease have normal immune systems that are subverted by *T whippelii* infection. Many microbes are known to alter the usual host cytokine response to infection. Epstein-Barr virus infection, for example, produces a protein similar to IL-10 that promotes a TH2 response,[10] and *Yersinia enterocolitica* blocks tumour-necrosis factor.[11] It is not unreasonable to hypothesise that *T whippelii* may also be capable of subverting the immune system by promoting IL-4 release or blocking macrophage-activating cytokines.

The approach used by Schoedon and colleagues offers hope for the eventual in-vitro propagation of other uncultivated or difficult-to-grow microbes, such as *Mycobacterium leprae* and *Treponema pallidum*. The expectation that all microbes can be propagated in pure (lifeless or cell-free) culture has diminished with our advancing knowledge of microbial ecology.[12] In the natural world, microbes tend not to exist as independent homogeneous populations, but rather as interdependent members of heterogeneous, complex communities interacting with other prokaryotic and eukaryotic cells. If we are to understand the microbial world that surrounds and sometimes infects us, it is time for our cultivation technology to move beyond the Petri dish to encompass complex multicellular culture systems.

For almost a century, the scientific community has awaited the successful cultivation of the Whipple bacillus. Today, we eagerly await answers to old and new questions about this microorganism and its mechanisms of pathogenesis. Our enthusiasm is tempered only by a patience wrought by 90 years of historical perspective.

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Genetic determination of bone density

An archaeological study of human skeletons from the 9th to 16th centuries has recently revealed an association between formation of bony osteophytes at joint margins and formation of bony outgrowths at sites of insertion of ligaments and tendons into bones (enthesophytes).[1] J Rogers and colleagues therefore suggest that the 20–25% of skeletons that exhibit both osteophytes and enthesophytes may reflect a group of individuals who can be classified as "bone formers".[1]

Many of the bone formers also had evidence of diffuse idiopathic skeletal hyperostosis (DISH). Although the aetiology of DISH is unknown, it is commoner in individuals with diabetes mellitus than in the rest of the population.[2] DISH is also associated with heterotopic ossification, the commonest complication of joint-replacement surgery.[3] Rogers et al suggest that the association between osteophytes, enthesophytes, and DISH may reflect genetic differences between individuals.[1] Alternatively, the association may also reflect a common mechanism of formation of these bone outgrowths in response to mechanical forces and/or diseases such as osteoarthritis or spondylarthropathies. In support of the hypothesis that genetic differences may account for variations in bone structure, twin and family studies of modern individuals have shown that 60–80% of the variation in bone density between individuals is genetically determined.[4,5] However, the genes that are responsible for regulating bone density have not been identified.

The candidate-gene approach has been used to examine the effect of allelic variation in several genes that are thought to play a role in normal bone homoeostasis. In this regard, the best-studied candidate gene encodes the vitamin D receptor (VDR).

Although some studies have reported a strong association between specific VDR alleles and bone density, other studies have found no association.[4–6] These disparate results are most likely to be due to the different genetic and environmental backgrounds in the cohorts in which the VDR was studied. Several other candidate genes that may regulate bone density, either independently or in combination with the VDR, are listed in the table.

An alternative to the candidate-gene approach is use of linkage analysis to identify genes responsible for variations in bone density between strains of mice[7] or in human hereditary disorders. For example, the loci for both the osteoporosis-pseudoglioma syndrome and a very high bone mass trait have recently been linked to human chromosome 11q12–13.[8,9] Since these two disorders have opposite effects on bone density, they may reflect mutations in the same gene, with osteoporosis-pseudoglioma syndrome being an autosomal recessive loss-of-function mutation and high bone mass being an autosomal dominant gain-of-function mutation. Cloning of the responsible gene(s) is likely to provide insight into the processes that regulate bone density. Identifying the genes that have the greatest impact in determining bone density may lead to improved screening, diagnosis, and

Gene	Type of studies	References
Receptors		
Vitamin D receptor	Allele association	Reviewed in Eisman,[4] Peacock,[5] Ralston[6]
Oestrogen receptor	Allele association	Reviewed in Ralston[6]
	Gene mutation/ deletion	Reviewed in Korach et al. *Recent Prog Horm Res* 1996; **51:** 159–86
Calcium receptor	Allele association	Cole et al. *J Bone Miner Res* 1997; **12** (S1): 257
	Gene mutation	Pollak et al. *Cell* 1993; **75:** 1297–303
Calcitonin receptor	Allele association	Masi et al. *J Bone Miner Res* 1997; **12** (S1): 257
β_3 adrenergic receptor	Allele association	Matkovic et al. *J Bone Miner Res* 1997; **12** (S1): 257
Cytokines, hormones, and antagonists		
Interleukin-6	Allele association	Reviewed in Ralston[6]
	Gene deletion	Reviewed in Greenfield et al. *Cells Materials (in press)*
Transforming growth factorβ	Allele association	Reviewed in Ralston[6]
	Gene deletion/ overexpression	Geiser et al. *J Bone Miner Res* 1996; **11** (S1): S378; Erlebacher et al. *J Cell Biol* 1996; **132:** 195–210.
Insulin-like growth factor-I	Allele association	Hosoi et al. *J Bone Miner Res* 1997; **12** (S1): 494
Parathyroid hormone	Allele association	Hosoi et al. *J Bone Miner Res* 1997; **12** (S1): 494
Interleukin 1 receptor antagonist	Allele association	Keen et al. *J Bone Miner Res* 1997; **12** (S1): 256
Bone-matrix proteins		
Type I collagen	Allele association	Reviewed in Ralston[6]
	Gene mutation	Reviewed in Ralston[6]
Osteocalcin	Gene deletion	Reviewed in Ralston[6]
α_2HS glycoprotein	Allele association	Dickson et al. *Bone Miner* 1994; **24**: 181–88
Other		
Apoprotein E	Allele association	Shiroki et al. *J Bone Miner Res* 1997; **12**: 1438–45
HLA markers	Allele association	Tsuji et al. *J Bone Miner Res* 1997; **12** (S1): 494