Microbe Dependence of *Mycobacterium leprae*: A Possible Intracellular Relationship with Protozoa

TO THE EDITOR:

Kato has postulated that *Mycobacterium leprae* may be dependent on concomitant microbes for its growth ([3, 4]). We offer an extension of this hypothesis by postulating that the original host of this mycobacterium may have been an amoeba or other free-living protozoan. The human (and armadillo) macrophages, which have certain features in common with amoebae, may thus be alternative host cells. This possibility was raised by one of us (TR) who was intrigued by the paradox that *Legionella pneumophila* has very fastidious growth requirements yet lives in water. Investigations showed that this bacterium survived and multiplied within amoebae ([7]). Of particular relevance is the fact that *L. pneumophila* replicates within human macrophages, and parallels have been drawn between infection by this pathogen in immunocompromised patients and lepromatous leprosy ([1]). Further, the more fastidious *Legionella micdadei* (Pittsburgh pneumonia agent) can in tissue be as acid-fast as *M. leprae* ([5]), suggesting a behavioral similarity.

We studied the interaction at 30°C between *M. avium* and an endosymbiont-carrying strain of *Acanthamoeba polyphaga* (Ap-1) which has previously been successfully used to isolate, via amoebal enrichment, *L. pneumonphila* serogroup-1, *L. pneumophila* serogroup-3 and a *Legionella*-like amoebal pathogen (LLAP-3) from human cases of pneumonia ([7]). Endosymbionts are bacteria that lie within the amoebal cytoplasm, replicate together with the host cell, and cannot be cultivated *in vitro*, indicating a close mutual dependence ([6]). Intriguingly, some human sera contain antibody to the small, curved, gram-negative, non-acid-fast endosymbiont.

A PYG broth culture of the amoebae was inoculated with a recent clinical isolate of *M. avium* in a bacilli-to-amoeba ratio of 10:1. Half of the mycobacteria were phagocytosed within 1 hr, and virtually all within 18 hr. After 3 weeks, all amoebae contained clumps of strongly staining, intact, acid-fast bacilli. The amoebae were subcultured every 3 weeks to a total of seven subcultures, and were found to replicate at the same rate as uninfected controls. During this time, all amoebae contained the clumps of acid-fast bacilli. Amoebae that became distended with bacilli appeared to be able to liberate these into the medium.

These studies showed that *M. avium* was readily phagocytosed by the amoebae, and appeared to establish a relationship with the host cell. This leads us to postulate that *M. leprae* may likewise exist, or have evolved, in close association with protozoa, and we suggest three possibilities. First, *M. leprae* may replicate within certain species or strains of amoebae, as previously suggested by Jadin ([2]). Second, the intracellular growth of *M. leprae* may be dependent on the pres-

ence of endosymbionts. Third, *M. leprae* may itself be an endosymbiont.

In view of these possibilities, it would be of interest to examine protozoa in those environments where the earth-burrowing armadillos have been infected with *M. leprae*, as well as to attempt the infection of various protozoa with this pathogen in the hope of developing a "cell line" for drug-resistance studies and large-scale propagation.

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