Mycobacterium X Identified as *Mycobacterium avium intracellulare* (Probably Mixed with *M. leprae* in Early Subcultures)

TO THE EDITOR:

Several strains of mycobacteria were cultivable from *Mycobacterium leprae*-infected human and armadillo tissues in media containing straight-chain dimethylated *n*-alkanes as the sole source of carbon and energy. The primary cultures and early subcultures did not grow on Löwenstein or in Dubos media, but in the foot pads of mice produced a disease similar to that obtained following injection of host-grown *M. leprae* ([7, 8]). The later subcultures, however, maintained in our laboratory on tetradecane–agar media, were also cultivable on Löwenstein media. These results suggest that early subcultures might be mixed isolates of *M. leprae* and a cultivable strain of mycobacteria. Today, out of the 18 cultures of *Mycobacterium X* that we possess, there is only one in our collection (AD-92) which still does not grow on Löwenstein medium. The isolates, identified as *M. avium intracellulare* by standard biochemical tests, have identical characteristics. They appear as rough, nonpigmented, unbilicated colonies on tetradecane media and have the following characteristics on which classification can be based. Slow, nonpigmented growth develops on Löwenstein medium at 30°C and 37°C; 0 ± growth at 20°C; no growth was registered at 42°C. NO_3 reduction, thiophen-2-carboxylic acid hydrazide (TCH), β-glucosidase, catalase S.Q., Tween 80 hydrolysis, acid phosphatase, 5% NaCl tolerance, arylsulfatase (3–14 days), and urease reactions were negative. NAP, catalase 68°C, tellurite reduction, nicotinamidase, pyrazinamidase reactions were positive. Cultures were resistant to isoniazid (INH) (0.2 μg), p-aminosalicylic acid (PAS) (0.5 μg), ethionamide (20 μg), and sensitive to streptomycin (4 μg), rifampin (40 μg), ethambutol (2 μg), capriomycin (50 μg), and cycloserine (30 μg) (per ml, respectively).

Two cultures of *Mycobacterium X* were confirmed as *M. intracellulare* (Serotype 19) by an independent laboratory (Dr. A. Laszlo, Health and Welfare Canada, National Research Centre for Tuberculosis).

The frequent isolation of cultivable mycobacteria from *M. leprae*-infected tissues has constantly been the subject of discussion and controversy, and has often been blamed on experimental error or technical incompetence. Claims of cultivation of *M. leprae* and reports on cultivation of mycobacteria from leprosy-derived tissues have appeared frequently in the literature every year during the past century. The pertinent literature has been reviewed in the past ([17]),

and recently surveys of attempted cultivation were presented by Prabhakaran [14], Kato [6], and Draper [2]. The reviewers are in agreement that with advanced taxonomical technics at hand [1] the presented cultures were identified as belonging to one of the known mycobacterial species rather than identical to *M. leprae.*

David, *et al.* [1] studied 36 slow-growing strains of mycobacteria isolated from the tissues of leprosy patients. Most of the strains belonged to clusters of the *M. avium-intracellulare-scrofulaceum* (MAIS) group. The ICRC bacilli of Bapat, the FMR strain of Mahadevan, and 21 CAMS strains from Cao Songnian were members of the same cluster.

Some investigators involved in cultivation trials of *M. leprae* claim that they hardly ever find cultivable mycobacteria in leprosy-derived tissues. In a recent communication, Pattyn and Portaels [12] claimed, "We have never any trouble with mycobacteria contaminants." One year later the same authors [13] declared that "cultivable mycobacteria have been isolated in large numbers from 2 out of 4 armadillos previously infected with human derived *M. leprae.*" These observations of Portaels, Franken, and Pattyn are in full agreement with our findings that the obtained strains are "difficult-to-grow mycobacteria." They do not grow in the early cultures on Löwenstein medium, but belong to the MAIS complex. Several investigators brought experimental evidence that in special physical conditions, with appropriate substrates and growth factors (mycobactin), with heavy inoculum and special pretreatment of the inoculum, cultivable species of mycobacteria can be detected, isolated, and identified in a high proportion of leprosy-derived tissues.

Smith, *et al.* [15] found a significant prevalence of leprosy in wild Louisiana armadillos. Cultivable mycobacteria belonging to the MAIS complex were isolated from the tissues of 10 out of 17 leprosy-infected armadillos (58.8%). Only 11.8% of the noninfected animals harbored cultivable mycobacteria.

The available literature from review articles, personal communications, and collections in our laboratories show that close to 200 strains of cultivable mycobacteria have been found during attempts to cultivate *M. leprae.* An oversimplified and rationalized explanation is offered by the conventional microbiologist that mycobacteria are widely distributed in nature. If so, then why are the same slow-growing species of mycobacteria not found with similar frequency in the nonleprous armadillos compared to the close to 200 strains detected in leprosy-derived human and armadillo specimens? Another riddle which awaits explanation is the fact that, with very rare exceptions, isolated strains belong to a well-defined category in the wide range spectrum of mycobacteria, probably depending upon the geographical area or the epidemiological distribution of cultivable mycobacteria. Most of the reported cultivable isolates belong to the MAIS complex. Strangely enough, *M. lepraemurium* "seems to be a hard-to-cultivate strain of *M. avium*" [2] and has a "fibrillar capsule of polar glycopeptidolipid, characteristic of species related to *M. avium*" [2, 3].

The high incidence of cultivable strains of mycobacteria in leprosy-derived tissues is intriguing. Based on the predictions of Kanai and Kondo [5] "that the same species of microorganisms takes different ways of living depending upon different *in vitro* and *in vivo* environment," the "Janus-face" theory [6] was offered, advocating that characteristics of *in vivo*-grown *M. leprae* might undergo such species-specific changes during *in vitro* cultivation as distant relatives of the same family. According to Draper [2], "*M. leprae* seems to be a three-headed Cerberus rather than a two-faced Janus, this hypothesis remains to be disproved." The results of Godal, *et al.* [4] demonstrated that the leprosy bacillus has a close antigenic relationship to a fast-growing species of mycobacteria, and do not Stanford and Rook [16] advocate that the leprosy bacillus must be an environmental saprophyte? Is not the MAIS complex environmental saprophytes? Draper [2] offers the notion that "pathological features of leprosy create an environment favourable for the culturable species." Portaels, Franken, and Pattyn [13] propose several hypotheses concerning the presence of cultivable mycobacteria in leprosy-infected tissues. The hypotheses are logical, although more studies are necessary to define the relationship of the obtained cultures to other mycobacteria and to *M. leprae.* Kazda [9] detected the presence of

noncultivable acid-fast bacilli resembling *M. leprae* in the environment of former leprosy endemic areas. He raised the question whether these noncultivable mycobacteria "might be of significance as cofactor in the genesis of leprosy."

The frequent presence of mycobacteria in *M. leprae*-infected tissues has far-reaching implications, mainly because their presence is difficult to detect in the primary cultures and early subcultures, as evidenced in the experiments of Kato ([7, 8]) and Portaels, *et al.* ([13]). It is obvious from the history of the known cultures that the cultivable mycobacteria in the *M. leprae*-infected hosts are present in extremely small numbers. They are cultivable with difficulty, using heavy inocula. *M. avium* is known as hard to grow in the primary cultures. Obviously, considerably more (if not all) leprosy-infected hosts might harbor cultivable strains of mycobacteria than are reported in the literature. Consequently, attempts to cultivate *M. leprae* must be focused on media highly selective for *M. leprae,* without promoting the growth of the accompanying cultivable mycobacteria. This problem is complicated by the fact that *M. leprae* is the slowest of the slow growers and even the slow-growing secondary species will overgrow *M. leprae* in non-selective media. The question arises whether such a selective medium will ever be discovered. It is of interest that drug sensitivity of the cultivable isolates is close or identical to drug sensitivity of *M. leprae* in the host.

As food for thought and material for further discussion, I offer the possibility that the presence of cultivable mycobacteria in the leprosy-infected tissues might be an integral part of the etiological involvement of *M. leprae* in the disease and cultivable mycobacteria play the role of "feeders," by offering growth-promoting mycobactins, or mycobactin-like factors, to the multiplication of *M. leprae in vivo* and probably *in vitro.* This hypothesis is strengthened by the fact that Olitzki, *et al.* ([11]) reported that *M. leprae* multiplied in media poor in nutrients when in the presence of mycobacterial substances.

The susceptibility of the armadillo to natural and experimental infections with *M. leprae* is poorly understood. It is not clear whether cultivable mycobacteria are introduced into the host prior to, simultaneously or after the infection with *M. leprae.* Muñoz Rivas ([10]) isolated cultivable strains of mycobacteria from 30 out of 35 healthy armadillos and 2 out of 3 *M. leprae*-inoculated animals. He also cultivated saprophytic mycobacteria from almost all the foods ingested by armadillos. Smith, *et al.* ([15]) also reported that 11.8% of the healthy armadillos harbored cultivable mycobacteria. It is safe to state that these species of mycobacteria were cultivable from the healthy and leprosy-infected armadillos because the armadillo did not possess the defense mechanism to destroy the cultivable strains of mycobacteria. One might be tempted to suppose that infection with *M. leprae* occurs by natural or experimental transmission in the armadillo (and probably in susceptible humans) unable to destroy the previously or simultaneously introduced cultivable mycobacteria. This working hypothesis is based on the assumption that *M. leprae* might be dependent on factors produced by the concomitantly present "secondary" mycobacteria.

—Laszlo Kato, M.D.

The Salvation Army
Catherine Booth Hospital Centre
4375 Montclair Avenue
Montreal, Canada M4B 2J5

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