

CULTIVATION OF *M.leprae* AND THOSE WHO STUDY MYCOBACTERIA

EDITORIAL

As throughfully known, *M.leprae* was discovered by G.A. Hansen in 1873 and it was the first bacteria to be related to a human disease. *M.tuberculosis* was discovered only in 1882. However, *M.leprae* still shows characteristics of an special entity taking in account that, to date, it is not possible to cultivate *M.leprae* despite all other important bacteria causative of human disease have been already isolated in laboratory. Even *T.pallidum*, one of the most resistant was successfully cultivated in tissue culture in 1981 (Fieldsteel et al).

Hansen's disease (HD) is still afflicting million of people worldwide and its control has been delayed due to the obstinacy of this peculiar mycobacteria. At least, some progress has been achieved in the therapeutical field, initially with the sulphones and later on with other drugs such as rifampicin and clofazimine (presently successfully used in the WHO recommended MDT regimens), otherwise the world would continue to be under the rule of this mycobacteria and menaced by the lesions it provokes in the human body, mainly in peripheral nerves, without any perspective of success.

Regardless of the progress in the treatment of HD and the perspective of new and powerful drugs, the possibility of cultivation of *M.Leprae* would consist in a tremendous improvement in the actions to eliminate HD.

Attempts to grow *M.leprae* in laboratory has been many and not successful. Since its discover, many animals and even human beings were inoculated in the attempt to reproduce the disease, with no satisfactory results. This picture has changed after the systematization of clinical observation suggesting that *M.leprae* would have a predilection for environments with temperature

below the average body temperature (36,5 QC). As a result, Shepard introduced in 1960 the inoculation of *M.leprae* in the mouse foot pad. This model allowed the study of many characteristics of this bacillus, such as its low period of multiplication (13-14 days), the viability period out of the human body and the possible lack of different strains. This model also permitted the study of new drugs and its mechanism of action. Later on, it was used the thymectomized and irradiated mice (Rees, 1966), nude mouse, armadillos and mongabay monkeys. Among them, armadillos still play an important role since they can produce a considerable amount of bacilli for other research such as the search for a vaccine and studies to a better understanding of the bacillus. Actually, they can be regarded as a live culture for *M.leprae*. For this reason, after its purification many information could be obtained directly from *M.leprae*. Therefore, there is no more need to use information extrapolated from similar bacteria such as *M.tuberculosis*. In this sense, many new information were obtained such as the different phases of its metabolism, enzymatic reactions and, moreover, the pattern of its cell wall, which are essential information for the design of new drugs and to the attempts to obtain cultivation of the bacillus. Additionally, many important substances, unique to *M.leprae* or common to other mycobacteria were identified, such as the mycolic acids, chains of acetilmuramic acid and glutamic acid interconnected by amino acids and particularly, diamino pimelic acid, phenol-glycolipid with phthiocerol dimycocerosate (PDIM), the lipid-arabino-manan among others. The bacillary genome is presently under study and more than 25% of its constituents has been already mapped.

Despite all this knowledge, the bacillus remains non-cultivable in laboratory. It is possible that some forms of acid fast bacilli isolated from patient are, in the reality, *M.leprae* showing a pleomorphism not found in other mycobacteria. In the other hand, it could be possible that its adaptation to the human body is to such an extent that lead to the blockage of production of some factor essential to its development. Conversely, one should remember that, in multibacillary patients, only a small proportion of bacilli remain viable even before treatment, what lead to the conclusion that the above mentioned adaptation to the human body could not be so extensive. This small number of viable bacilli could be explained by the lack of some enzymes such as catalase and a deficiency of superoxide dismutase (SOD).

Mycobacteria shows some particularities that are unique among bacteria. They are acidfast staining with Ziehl-Neelsen method, present a cell wall with high content of lipids and are omnipresent. They can be found in any environment such as the fungus. They are saprophytes, but more correctly saprobious, most of the time, but can parasite invertebrates such as snail, fishes, reptiles, amphibious, birds and mammals. Many cattle diseases are caused by mycobacteria. They are also responsible for many human diseases such as the infections by *M.tuberculosis*, *M.marinum* and *M.kansasii*. Presently, they are regarded with increasing importance since, even being considered saprophytes, they are being blamed for causing many diseases in the immunological deprived

patient, due to drugs or diseases such as cancer and infections by HIV.

Mycobacteria present many antigens as a common feature and each one shows some particularity regarding cultivation, such as an specific and essential temperature for growth or some specific growth factor which are essential to its development.

There are many things to learn from the study of mycobacteria, as a whole, which could be applied to the attempts to cultivate *M.leprae*. However, there is a lack of interest of researchers in this area, which is essential to hansenology. One reason to this could be the discouraging final results of many studies which were initially very promising. Not long ago, Skinsnes published a series of very interesting articles which suggested that the cultivation of *M.leprae* was attained. However, his findings were not confirmed and the mycobacterium this author isolated from patients was, most probably, the *M.scrofulaceum*.

It would be very important that Brazilian microbiologists, mainly those involved in the study of mycobacteria, could overcome the prejudice against *M.leprae* and start moving their research towards this mycobacterium.

In this sense, in my opinion, our journal should encourage the publication of articles related to mycobacteria as a whole. I am sure that this suggestion will be supported by all members of the Brazilian Association of Hansenology and the College of Hansenology of the Endemic Countries.

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