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TAXONOMIC STUDIES ON THE LEPROSY BACILLUS

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The taxonomy of nonculturable bacteria presents many problems, and the relatively small amount of information about the leprosy bacillus available 102 years after its discovery reflects this. The discovery of the armadillo as an in vivo culture system has made available for the first time large numbers of leprosy bacilli, but even so we have few taxonomic tools suitable for the investigation of organisms inside tissues. This paper gives an account of attempts to get round the special problems involved and of the results that have been achieved (1). The demonstration of a close antigenic relationship between the leprosy bacillus and a culturable nonpathogenic organism would obviously provide a simple way to manufacture a vaccine on a large scale.

A study of the literature provides some important taxonomic information. If one accepts that possession of mycolic acids of the mycobacterial type is proof of generic station, then the work of Etemadi and Convit (2) shows the leprosy bacillus to be a Mycobacterium.

Secondly, skin-testing with tuberculin PPD does not distinguish between tuberculoid and lepromatous leprosy, although this somewhat degraded antigen cross-reacts strongly with all other members of the slow-growing subgenus.

Therefore, the leprosy bacillus is a fast-grower or something distinct. The work of Godal and his colleagues (3) has demonstrated that the leprosy bacillus has a greater antigenic relationship to M. duvalii, a fast-growing species, than to a number of other species including several slow-growers.

Since the immune defect in lepromatous leprosy shows some specificity (3) one can use the patient himself as a tool for the investigation of the phylogenetic relationships of his own parasite by looking for organisms to which the tuberculoid patients give positive skin-test responses while the lepromatous ones do not. Reagents suitable for such investigation can be made from ultrasonic lysates of living mycobacteria (4), which have much greater specificity than PPD's.

Nineteen such reagents were prepared, and these have been used to test tuberculoid and lepromatous leprosy patients, and healthy leprosy contacts in East Africa (5) and Burma. The percentages of positive reactors to the different reagents were recorded.

With the exception of M. gordonae which showed a depression in the lepromatous patients, no members of the serologically defined, slow-growing subgenus gave the relevant reactions, whereas almost all the fast-growers showed a depression in the lepromatous patients. Organisms of the M. vaccae group demonstrate reproducibly both the immune defect and crossreactivity with the leprosy bacillus (Fig. 1).

Turning from the field to the laboratory, ultrasonic extracts of leprosy bacilli obtained from human tissues and from tissues of eight-banded and nine-banded armadillos have been analyzed by immunodiffusion with immune rabbit sera and with sera from lepromatous patients.

Twelve antigens have been demonstrated in the extracts of M. leprae (Fig. 2), 6 of these fall into group i (i.e. antigens shared by all mycobacteria and in part by other genera [6]), and 4 belong to group iv (species-specific). The position of the other 2 is not certain. The notable thing about the leprosy bacillus is its lack of groups ii and iii antigens which are characteristic of slow-growing and fast-growing species respectively. The deficiency appears to be shared with the M. vaccae group of organisms and with the Gordona species of Tsukamura. However, since the latter do not possess mycolic acids of the mycobacterial type and skin test reagents prepared from them do not give the reactions we have discussed, we probably need not consider them further. There is no reason to link the leprosy bacillus with any of the coryneform or other related genera, which possess no more than three group i antigens.

Studies of cellular immunity in mice infected or immunized with M. leprae also indicate a relationship with fast-growing species and especially with the M. vaccae group. As an example of such experiments, groups of young BALB/C mice were immunized with 10^6 leprosy bacilli and kept in the same cages as unimmunized controls. After 5 and 6 months the mice were killed and lymphocyte transformation tests were carried out on their lymph node cells using a battery of antigens from 11 species of mycobacteria.

The results are expressed as the response to each antigen in immunized mice, minus the response to the same antigen in control mice. Thus the effect attributable to the leprosy bacillus is clearly seen (Fig. 3).

After 5 months, cells from immunized mice showed enhanced responses to all the environmental species to which control cells were sensitive. This effect, which is also seen in man after BCG and in mice after injection of ultrasonicated mycobacteria, is probably due to an adjuvant effect or to weak cross-reaction. However, it is transient and when tested at 6 months, only the responses to M. vaccae and the leprosy bacillus were above control values.

In another experiment mice were immunized with 10^7 M. vaccae organisms or BCG organisms into the foot pad, and foot pad tests were performed with reagents prepared from M. vaccae and M. leprae, one and two months later. M. vaccae immunized animals gave positive responses to both antigens, whereas

Table 1. List of organisms from which skin-test reagents were prepared.

M. avium	*M. chelonei*
M. gordonae	*M. diernhoferi*
M. kansasii	*M. duvalii*
M. marianum	*M. flavescens*
M. marinum	*M. fortuitum*
M. tuberculosis	*M. gilvum*
M. ulcerans	*M. nonchromogenicum*
M. xenopi	*M. smegmatis*
*M. A** (not yet named)	*M. vaccae*
M. leprae	

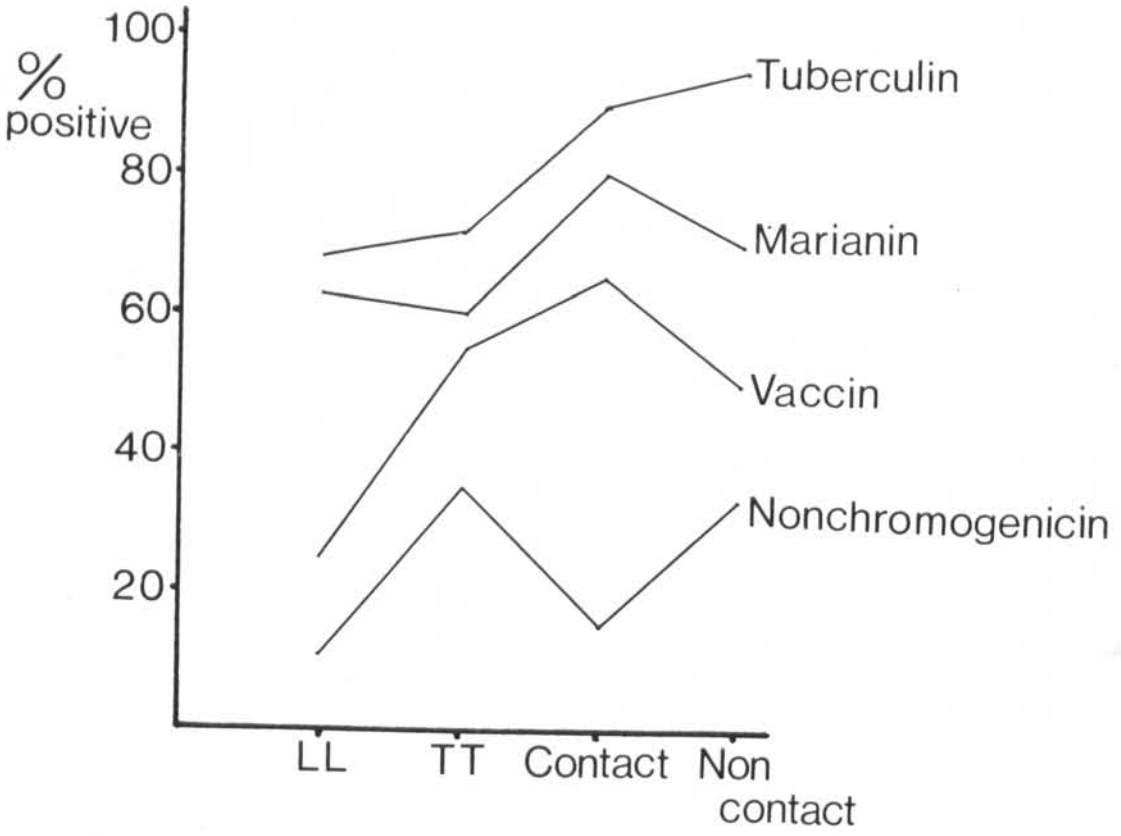

FIG. 1. Diagram showing the relative percentages of positive reactors to four skin test reagents in lepromatous and tuberculoid leprosy patients, their contacts and the normal population (called noncontacts).

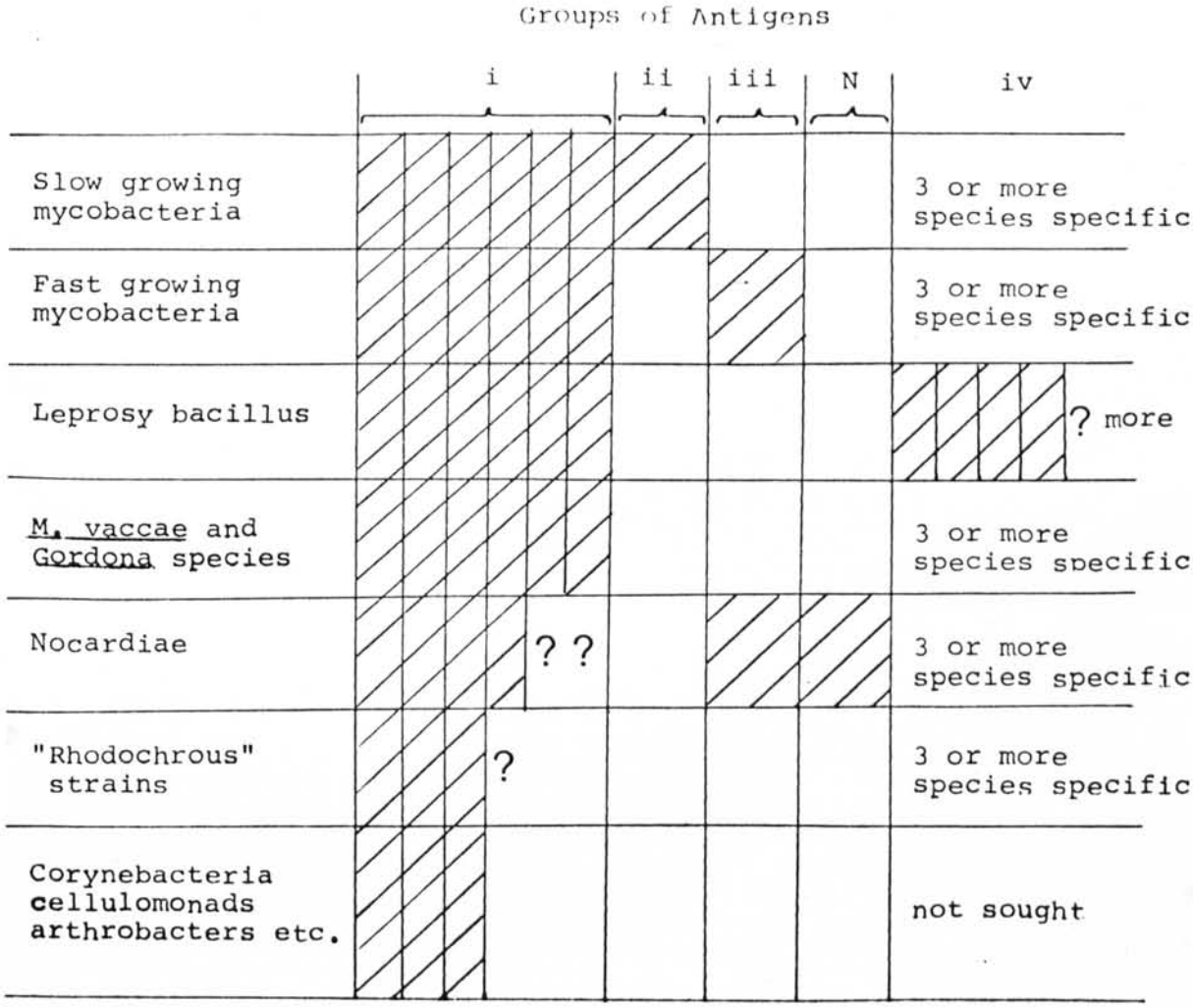

Fig. 2. Schematic diagram showing the antigenic relationships established by immunodiffusion analysis between the leprosy bacillus, other mycobacteria, nocardiae and coryneform genera. The antigenic grouping is according to Stanford (1973). Antigens are showed in the vertical columns where indicated by hatching. Group iv antigens are specific to individual species.

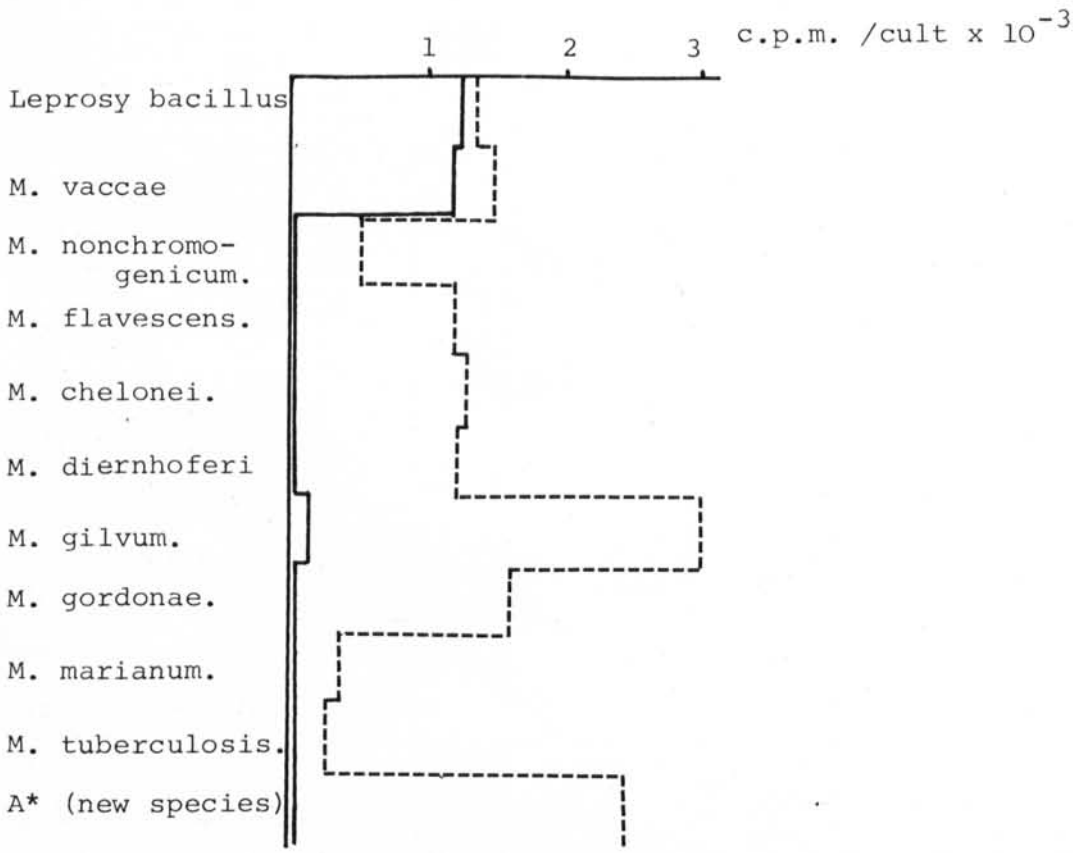

Fig. 3. Response to mycobacterial antigens of lymph node cells from mice immunized 5 (- - -) and 6 (———) months previously with 10^6 leprosy bacilli. Results are expressed as: c.p.m./culture in immunized mice - c.p.m./culture in unimmunized controls.

BCG immunized animals responded to neither, although positive to PPD. Similar experiments performed with M. smegmatis and M. neoaurum gave negative responses to M. leprae antigen.

In view of the strong immunological evidence linking M. leprae with M. vaccae and some other fast-growers, we sought nonimmunological approaches to the problem. Two tests have been reported in the literature as specific to the leprosy bacillus, the extraction of acid fastness by pyridine, a reaction stressed by Fisher and Barksdale (7) and the DOPA oxidase test of Prabhakaran and Kirchheimer (8). We have found both tests beset with practical difficulties.

Some fast-growing organisms, and, in particular, member of the M. vaccae complex, have given positive results with the DOPA oxidase test, but this has not been consistent. However, pyridine does consistently extract the acid fastness of some members of this group. It seems probable that neither of these tests is specific for the leprosy bacillus, but that they add to the evidence for a close relationship with certain fast-growing mycobacteria.

Thus evidence from leprosy patients themselves, from immunodiffusion analysis, from the correlates of cell mediated immunity in experimental animals and from the available biochemical tests all link the leprosy bacillus with the fast-growing group of organisms, and perhaps most closely with the M. vaccae complex.

Leprosy is probably a recent disease. There is no evidence for its existence prior to 1000 B.C., whereas tuberculosis existed at least 25,000 years ago. Thus unless one believes in spontaneous divine creation, the leprosy bacillus is derived from a still existent progenitor. This progenitor is most unlikely to be a pathogen since none of those known, with the possible exception of the so-called "M. leprae bubulorum," resemble it. Thus it must be an environmental saprophyte.

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