THE ORIGIN OF NATURAL REACTIVITY TO LEPROMIN

THE ASSOCIATION BETWEEN THE MITSUDA REACTION AND REACTIONS TO GRADED DOSES OF TUBERCULIN [1]

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I. INTRODUCTION

Reactivity to lepromin has been shown by many workers to be common among healthy persons. For example, in our studies ([8, 11, 12]) on Mactan Island, Cebu, Philippines, the proportions of persons showing the late (Mitsuda) reaction have been very high: 23 per cent of children of from 6 to 35 months of age, 65 per cent of school children 7 to 9 years old, and more than 90 per cent of adults. In these studies the lepromin used was prepared by the Mitsuda-Hayashi method, and nodules 5 mm. or larger in diameter on the 21st to the 28th day were regarded as positive.

The principal theories which have been offered to explain the existence of reactivity to lepromin are: (1) prior infection with *Mycobacterium leprae;* (2) prior infection with *M. tuberculosis,* and (3) prior infection with some other species of the genus Mycobacterium. It has also been suggested by Wade ([23, 26]) that the stimulus to reactivity may be the test dose of lepromin itself; that is, that a prior infection is not essential.

These theories are based on the view, generally accepted by leprologists, that the occurrence of the Mitsuda reaction is dependent upon the presence of intact leprosy bacilli in lepromin. Recently this view has been challenged. Kooij and Gerritsen ([17]) have reported a reaction, similar in all respects to the one of Mitsuda, following injection of a suspension of normal liver particles prepared as Dharmendra prepares lepromin but concentrated one hundred times.

The present paper deals only with the frequency with which reactivity of the Mitsuda type has been observed in various population groups; and, in particular, with the association between reactivity to lepromin and infection or opportunity for infection with *M. leprae,* and between reactivity to lepromin and infection with *M. tuberculosis.* New data are presented on the relationship between the lepromin and tuberculin

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reactions, which were observed in comparable groups of children tested with various dosages of tuberculin. The immunologic significance of the lepromin reaction is not discussed.

II. RELATIONSHIP OF REACTIVITY TO PRIOR INFECTION WITH *M. leprae*

The accepted proof of infection with *M. leprae* at the present time is the existence of a well-defined clinical syndrome in association with the presence of a noncultivable acid-fast bacillus in the tissues. This syndrome, in fact, is usually so definite as to lead to a presumptive diagnosis even when few or no bacilli can be demonstrated, as frequently occurs in the tuberculoid type. Infection of a subclinical nature may occur but cannot be proved, because *M. leprae* cannot be cultivated and its morphologic and staining characteristics are not sufficiently definitive to justify a diagnosis in the absence of clinical or histologic evidence.

In the lepromatous type of the disease, in which resistance of the body is overcome and vast numbers of bacilli are present, the result of the lepromin test is nearly always negative. This phenomenon is often described as a type of specific anergy, but the explanation is unknown.

According to most authors, the lepromin reaction is usually positive in the tuberculoid type. In an unpublished study made on Mactan Island, Guinto tested 54 children exhibiting tuberculoid macular lesions and found 80.1 per cent to show the late lepromin reaction. Of 906 healthy noncontacts of equivalent ages only 48.2 per cent were positive. Further comparisons between patients and the general population from which they are drawn are necessary. If the greater reactivity of persons suffering from tuberculoid leprosy can be established, it would be the best obtainable evidence that natural infection with *M. leprae* causes reactivity of the Mitsuda type. In such a study, obviously, the type diagnosis must be made without knowledge of the result of the lepromin test.

Judging from secondary attack rates, persons living in household association with lepromatous leprosy must have a much greater opportunity for infection than those exposed to cases of the tuberculoid type; and the latter, in turn, are somewhat more exposed than the remainder of the population. Our experience in Cebu as reported by Guinto *et al.* ([13]) indicates that there is little difference between these three classes in respect to reactivity to lepromin. The percentages of persons of all ages who reacted were: for contacts of lepromatous patients, 73.4; for contacts of tuberculoid patients, 68.3; and for persons not known to have lived in household association with persons suffering from either type, 68.2. It was observed, however, that in the 10 to 14 years age group the frequency of reactors was highest among contacts of lepromatous patients. Further data on the reactivity of children exposed to each type of leprosy in the household should be obtained.

In India, Dharmendra and Jaikaria ([7]) compared the frequency of late lepromin reactions in an endemic area in West Bengal with that in districts of the Punjab where leprosy was uncommon. The rates for all ages, adjusted for age differences, were 61.9 per cent for the endemic area and 32.7 per cent for the nonendemic area. There may, however, have been other environmental and possibly indeterminable differences of a significant nature which were unknown to the authors.

Thus infection with *M. leprae* may produce reactivity. It is, however, not a prerequisite. A number of workers have reported such reactivity to be frequent in places where leprosy is absent or extremely rare ([1, 2, 4, 6, 9, 15, 21]).

III. RELATIONSHIP TO INFECTION WITH *M. tuberculosis*

In the search for a nonspecific origin of reactivity to lepromin, suspicion falls first upon *M. tuberculosis*. Active tuberculosis has long been considered one of the principal complications to which a patient with lepromatous leprosy is subject; in fact, it was formerly a leading cause of death in such patients. Reactions to small doses of tuberculin and also local reactions following BCG vaccination are very frequent among patients with lepromatous leprosy, but whether they are more or less frequent than in the same age groups of the general population is an unsettled question.

There are many reports of positive correlation between reactivity to tuberculin and to lepromin when the tests are performed simultaneously in apparently healthy persons. These reports, together with the fact that BCG vaccination will induce reactivity to lepromin in healthy children, constitute the principal support for the theory that prior infection with *M. tuberculosis* causes reactivity to lepromin.

In one of our studies on Mactan Island, related to the effectiveness of BCG in producing reactivity to lepromin, reported by Doull *et al.* ([8]), a pertinent observation was made. It was found that of 483 children, from 6 to 35 months of age, living in their homes and considered to be healthy, only 2.3 per cent reacted to 0.0001 mgm. of PPD (5 TU) at the beginning of the study. Because of their young ages, any tuberculous infection in these children would obviously be recent. Therefore, insofar as a small dose of tuberculin is an indicator, this group of children was relatively free from tuberculous infection. Nevertheless, as noted above, 23.2 per cent reacted to lepromin. This seems definitely to prove that reactivity to lepromin may be gained naturally in the absence of infection with *M. tuberculosis*. In these children, infection with *M. leprae* was also highly improbable. There were no cases of leprosy in any of the families concerned, and the prevalence rate in the general population was well below 2.0 per cent for all forms of the disease.

IV. PRIOR INFECTION WITH SOME OTHER SPECIES OF MYCOBACTERIUM

Chemical analysis indicates that several members of the genus Mycobacterium resemble one another, qualitatively, in chemical composition. Quantitatively, there are differences. For example, the total lipid content is much higher in the human tubercle bacillus than in the avian and bovine types and in the timothy grass bacillus. The polysaccharide content, on the other hand, is higher in this saprophyte than in the three types of tubercle bacillus. The various species cannot be identified by serologic reactions, although certain broad groups can be differentiated. As regards skin sensitivity elicited by intradermal injection of "tuberculins" made from different species, the experiments of Green ([10]) in guinea-pigs and of Johnson *et al.* ([16]) in cattle have shown that animals sensitized with one species of Mycobacterium will react to PPD prepared from another but that larger doses are required of heterologous than of homologous PPD.

There is also epidemiologic evidence which suggests that response to small doses of tuberculin indicates infection with *M. tuberculosis* but that reactions only to large doses may be nonspecific. In studying the results of tuberculin tests performed on young women in 76 nursing schools throughout the United States, Palmer ([20]) noted that response to 0.0001 mgm. of PPD (5 TU) became more frequent as the degree of previous contact with tuberculosis increased, whereas the frequency of reactors to 0.005 mgm. of PPD (250 TU) was independent of the degree of contact but closely related to place of residence. He concluded that a low sensitivity to tuberculin brought out only by large doses (1 mgm. of OT or 0.005 mgm. of PPD, for example) apparently represents infection by a different organism with a different mode of transmission. He commented that the latter (unidentified) organism must be antigenically related to the tubercle bacillus, highly prevalent in certain geographic areas, and apparently nonpathogenic for human beings.

In an effort to throw further light on the relationship between the tuberculin and lepromin reactions, Guinto *et al.* ([11]) arranged their results and those obtained by others in ascending order of the strength of tuberculin used. It was thought that the relationship between the two types of reactivity might be closer with high than with low doses of tuberculin; that is, the difference between the proportions reacting among tuberculin positives and negatives, respectively, would be expected to increase as the dosage of tuberculin is raised. Reactions to large doses would include both specific reactions to tuberculoprotein and nonspecific responses to such mycobacterial proteins as may be present in both the tubercle bacillus and other mycobacteria. The comparison was recognized to be an unsatisfactory one, because different tuberculins and lepromins were used. These data, supplemented by some published more recently, are summarized on Table 1.

Inspection of Table 1 shows a higher frequency of reactivity to lepromin in tuberculin-positive persons than in tuberculin negatives in all the reports. This is the crucial comparison. The difference was least (22%), i.e., the relationship was poorest, in a study ([11]) in which the dose of PPD was only 0.00002 mgm. (1 TU). With higher doses, there is no obvious trend to larger differences as the dosage was increased. Two authors, de Souza Campos *et al.* ([22]) and Chaussinand ([4]), used OT in a dosage regarded as equal to about 1,000 TU of PPD. At this strength, practically all tuberculin positives responded to lepromin, but many of the tuberculin negatives did also.

TABLE 1.—*Frequency of the late lepromin (Mitsuda) reaction in tuberculin-positive and tuberculin-negative persons simultaneously tested with both antigens, by various authors, according to dose of tuberculin used.*

Reference	No. tested	Ages (yrs.)	Approx. dosage tuberculin (TU)	Lepromin positive, per cent				
				Tuberculin Positive	Tuberculin Negative	Difference (%)	SE of Diff. (%)	Diff.[a] SE
(11)	544	7-9	1	84.2	62.2	22.0	5.9	3.7
(18)	287	5-8	5	51.1	19.8	31.3	7.0	4.5
(18)	334	9-16	5	81.3	46.9	34.4	5.3	6.5
(5)	313	all	10	92.5	55.2	37.3	5.6	6.7
(18)	278	adults	50	88.3	49.1	39.2	5.9	6.6
(19)	287	5-8	100	37.1	12.4	24.7	5.7	4.8
(19)	334	9-16	100	69.5	26.9	42.6	7.3	5.8
(7)	260	all	100	53.7	15.3	38.4	5.9	6.5
(11)	544	7-9	250	77.5	34.0	43.5	4.5	9.7
(22)	148	2-16	1000	100.0	54.7	45.3	9.7	4.7
(4)	231	1-8	1000	95.0	19.0	76.0	6.4	11.9

[a] In random sampling a difference as great or greater than 2 times SE in either direction is expected about 5 times in 100; of 3 times SE about 3 times in 1000.

It seemed desirable to obtain further information by means of a special study, in which the same lots of tuberculin and of lepromin would be used for all the subjects tested. It was also decided that the lowest dose of tuberculin should be 0.0002 mgm. of PPD (10 TU), and the highest 0.004 mgm. (200 TU), a twenty-fold range.

A total of 1,004 school children, 6 to 10 years of age, living on Mactan Island, were selected for testing, and parental consent was obtained. These children were tested with lepromin and with PPD, the latter in a dosage of 0.0002 mgm. (10 TU). For various reasons 136 children dropped out at this stage. Those who dropped out did not differ significantly as regards reactivity to tuberculin or lepromin from those who

remained in the study. The analysis is therefore restricted to the results in the 868 children who completed the experiment.

Of the 868, 237 or 27.3 per cent reacted to tuberculin (10 TU) and 631 did not. Of the tuberculin reactors, 89.9 per cent also reacted to lepromin, and of the nonreactors, 72.3 per cent, an excess of 17.6 per cent for the reactors. This difference is a highly significant one in the statistical sense, being 5.5 times its standard deviation.

The 631 children who were negative to 10 TU were divided at random into seven subgroups. Each of the subgroups was tested with a higher dose of the same PPD, as follows: 0.0004 mgm. (20 TU), 0.0006 mgm. (30 TU), 0.0008 mgm. (40 TU), 0.001 mgm. (50 TU), 0.002 mgm. (100 TU), 0.003 mgm. (150 TU), or 0.004 mgm. (200 TU). Successively higher doses were not given to any subgroup; the result for only one higher level was obtained for each.

Considerable variation occurred in the frequencies of lepromin positives among the tuberculin positives and negatives of these subgroups, and the percentages were therefore smoothed somewhat by the method of moving averages. The figures for the subgroups tested with 20, 30, and 40 TU were combined; also those for 30, 40, and 50 TU; 40, 50, and 100 TU, etc. The essential features of the results are shown in Table 2.

TABLE 2.—*Frequency of the late lepromin (Mitsuda) reaction in tuberculin-positive and tuberculin-negative school children 6-10 years of age, Mactan Island, Cebu, tested with the same lot of PPD, classified according to the dosage of tuberculin used.*

Dosage of PPD in TU	No. of children tested	Lepromin positive, per cent		Difference (%)	SE of Diff. (%)	Diff.[a] SE
		Tuberculin				
		Positive	Negative			
10	868	89.9	72.3	17.6	3.2	5.5
Negative to 10[b]						
20, 30 or 40	275	87.2	71.5	15.7	7.0	2.2
30, 40 or 50	264	81.0	73.1	7.9	6.3	1.3
40, 50 or 100	271	82.5	67.0	15.5	5.9	2.6
50, 100 or 150	268	82.9	61.1	21.8	5.7	3.8
100, 150 or 200	271	81.9	60.1	21.8	5.5	4.0
150 or 200	177	80.0	59.7	20.3	6.9	2.9

[a] See footnote, Table 1.

[b] Note that percentages are moving averages for 20, 30 or 40; 30, 40 or 50, etc.

Inspection of Table 2 shows that the positive correlation between the two reactions which was observed at the 10 TU level, was also present in each subgroup of negatives when tested with a higher dose. The differences between tuberculin positives and tuberculin negatives

in respect to the frequency of lepromin reactors, however, do not show any strikingly upward trend with increased doses of PPD, although they are somewhat greater at higher than at lower levels.

DISCUSSION

Of Mactan children of 7 to 10 years of age, those reacting to 10 TU of PPD tuberculin would be expected to include all, or nearly all, who had been infected with *M. tuberculosis*. These reactors, moreover, would not be expected to include many who were sensitive only to the "antigenically related" organism hypothesized by Palmer ([20]), although 10 TU of PPD tuberculin might conceivably contain sufficient of such a nonspecific antigen to produce reactions in some of these children. The frequency of reactivity to lepromin among tuberculin reactors exceeded that among nonreactors by 17.6 per cent. The only known difference between the groups is that one reacted to tuberculin and the other did not. If our premises are correct, the excess percentage of lepromin reactors in the positive group may be attributed to prior infection of these children with *M. tuberculosis*.

Considering as a unit all 631 children who were negative to 10 TU and retested with higher doses of PPD, that is, without regard to the subgroups shown in Table 2, 82.5 per cent of the tuberculin positives reacted to lepromin and 67.1 per cent of the tuberculin negatives. This excess percentage of lepromin positives among the reactors to higher doses of tuberculin (15.4), or most of it, may in turn be attributed to infection with some microorganism related antigenically to the tubercle bacillus.

The matter may be considered in another light. If there were no association whatever between the reactions to PPD at the 10 TU level and the reactions to lepromin, there would have been expected among the tuberculin positives about 183 who were also positive to lepromin. This figure is obtained by multiplying the proportion of lepromin positives for all the children, 77.1 per cent, by the total reacting to 10 TU of tuberculin, that is 237. The actual number of children reacting both to lepromin and to 10 TU of tuberculin was 213. Thus the number reacting to lepromin because of prior infection with *M. tuberculosis* would be about 30 (213 minus 183); or, if the variation expected in random samples of 237 be taken into account, would be expected to vary from a maximum of 48 to a minimum of 12.[3] The expectancy of 30 is only 4.5 per cent of the total number of lepromin reactors (669); the maximum (48) is 7.2 per cent, and the minimum (12) is 1.8 per cent.

Of the 631 children negative to 10 TU, and retested with higher doses, there were 456 or 72.2 per cent who were reactive to lepromin.

[3] In random sampling the chance of this difference exceeding 48 or being less than 12 is about 1 in 20.

There were 212 who were positive to one or another of the larger doses of PPD. If there were no association between the results of the lepromin and tuberculin tests, 72.2 per cent of the 212 tuberculin positives or 153 children would be expected to show positivity to lepromin. Actually, 175 were positive, an excess of 22. Again, viewed as a problem in random sampling, this difference might be expected to exceed 40 or be less than 4 not oftener than once in 20 trials. On this basis, the proportion of children who reacted to lepromin because of sensitivity attributed to infection with some species of Mycobacterium other than *M. tuberculosis* would be about 8.8 per cent at the most, 4.8 per cent on the average and 0.9 per cent at the least. That is, although a positive correlation was present both at 10 TU and higher levels, the reactivity of a maximum of only 16.0 per cent (7.2% + 8.8%) of the total lepromin reactors can be accounted for by the two hypotheses—i.e., specific infection with the tubercle bacillus and nonspecific mycobacterial infection—taken together. This leaves a tremendous proportion (84%) of the total reactors to be accounted for. If a dose of 150-200 units of PPD can be taken as sufficiently high to eliminate those previously infected either with *M. tuberculosis* or the hypothetical mycobacterium, it is clear that mycobacterial infections do not provide a satisfactory explanation, because 60 per cent of children negative to that dosage nevertheless reacted to lepromin (Table 2).

In a previous study of Mactan school children made by Guinto *et al.* ([11]), in which the lots of PPD and of lepromin were different from those used in the present study, a preliminary dose of 0.00002 mgm. (1 TU) of PPD was followed, in the negatives, by a dose of 0.005 mgm. (250 TU). By a similar computation to that used above it has been estimated that the average percentages of total lepromin reactors who were reactive because of prior infection with *M. tuberculosis* (taking 1 TU as the discriminating dose) was only 3.9 per cent; because of infection with some other species of Mycobacterium, 14.8 per cent, and on both of these hypotheses taken together, 16.1 per cent.

These facts, together with the occurrence of reactivity in persons whose opportunity for infection with *M. leprae* is very remote, make it necessary to take a broader view of the origin of lepromin reactivity of the Mitsuda type.

Among the suggestions that have been made is that the Mitsuda reaction is the result of sensitization caused by some antigen present in the test dose itself. Wade ([24]) has stated: "It would seem (as noted by Rodriguez) that the elements that are directly concerned in the production of the reaction lesion may not be formed until after the antigen is introduced." At present this hypothesis cannot be proved or disproved because there is no serologic or other method by which an individual who will show lepromin positivity can be distinguished from one who will not. If sensitization by the test dose is the major factor which causes the Mitsuda lesion to appear two or three weeks later, it is necessary to assume—as is necessary also with the theory of Kooij

and Gerritsen ([17])—that the capacity to respond must increase as children grow older. In infants, the reaction is usually negative—as was noted many years ago by K. R. Chatterjee ([3])—but thereafter, judging from our experience on Mactan Island ([8]), the frequency increases very rapidly as age advances. Guinto and Wade ([14]) have presented data suggesting that the frequency of reactivity to lepromin may vary with the environmental background, being more frequent in the countryside than in the town. It would appear necessary to assume that capacity to respond varies also with the environment in order to meet the hypothesis of sensitization by the test dose. Although further evidence is needed regarding this matter, the possibility of some underlying environmental factor cannot be denied. The evidence brought forward in the present report suggests only that this factor is probably not prior mycobacterial infection.

In our Mactan experience ([8]) another observation was made which throws doubt on the sensitizing capacity of the test dose as a major factor. Comparison was made between lepromin reactivity (Mitsuda) of a group of retested children, who had been tested five months earlier, with that of a comparable group not given an initial lepromin test. The difference was small and of doubtful statistical significance, indicating that the initial test had not been materially effective in raising the level of reactivity. As far as we are aware, this is the only controlled study of the subject which has been reported.

SUMMARY AND CONCLUSIONS

This study deals with lepromin reactivity of the Mitsuda type, observed in apparently healthy children on Mactan Island, Cebu, Philippines. The principal theories which have been offered to explain this type of reactivity are prior infection with *M. leprae,* with *M. tuberculosis,* or with some other species of mycobacteria. It is improbable that the majority of these children, and especially those under three years of age, could have been exposed to leprosy. Although there is a positive correlation between reactivity to tuberculin and that to lepromin, the excess of lepromin reactors among tuberculin positives over the number expected if there were no association comprises a very small fraction of the total number reacting to lepromin. This is true both for small doses of tuberculin, reaction to which is regarded as specific for infection with *M. tuberculosis,* and for larger doses which may indicate prior infection with other species of Mycobacterium. This suggests that lepromin reactivity among these children is caused in most instances by some factor other than infection with *M. tuberculosis* or any related species. The theory that the test dose of lepromin is the responsible sensitizing factor is not in itself adequate as far as can be judged from the effect of a second lepromin test in a controlled study. This theory requires also the assumption of an increasing capacity to

respond as age advances and perhaps the assumption of variation in capacity to respond in different environments.

Further studies of lepromin reactivity are necessary. Except for nonreactivity in lepromatous leprosy, which may have some other explanation, there is little evidence that reactivity has any relationship to infection with *M. leprae*. There is need for confirmation of the usually-accepted opinion that patients with tuberculoid leprosy are almost universally positive. Comparison should be made with healthy siblings and the groups should be matched carefully in respect to age and sex. Further evidence should be sought also concerning the theory that increasing frequency of reactivity with age is simply a "maturation" phenomenon. This might be obtained by testing, in various areas, groups of children differing in respect to specified environmental conditions but comparable in other respects.

RESUMEN Y CONCLUSIONES

Versa este estudio sobre la reactividad, tipo Mitsuda, a la lepromina, observada en niños aparentemente sanos en la isla de Mactan, Cebú, Filipinas. Las principales teorías ofrecidas para explicar esta forma de reactividad son infección anterior por el *M. leprae*, por el *M. tuberculosis* o por alguna otra especie de micobacteria. Es improbable que la mayoría de estos niños, y en particular los de menos de tres años, puedan haber estado expuestos a la lepra. Aunque existe una correlación positiva entre la reactividad a la tuberculina y la reactividad a la lepromina, el exceso de reactores a la lepromina entre los positivos a la tuberculina sobre el número esperado si no hubiere asociación comprende una fracción muy pequeña del total de los que reaccionan a la lepromina. Esto es un hecho tanto para las dosis pequeñas de tuberculina, una reacción a las cuales se considera como específica para infección por el *M. tuberculosis*, cuanto para las dosis mayores que pueden indicar infección anterior por otras especies de micobacterias. Sugiere esto que la reactividad a la lepromina entre estos niños es ocasionada en la mayor parte de los casos por algún factor distinto de la infección por el *M. tuberculosis* o alguna especie afín. La teoría de que la dosis de prueba de lepromina es el factor sensibilizador causante no resulta por sí misma adecuada en lo que cabe juzgar por el efecto de otra dosis de lepromina en un estudio fiscalizado. Requiere además esta teoría la suposición de que aumenta la capacidad para reaccionar a medida que avanze la edad y quizás también la suposición de que varia la reacción en distintos medios.

Se necesitan más estudios de la reactividad a la lepromina. Excepto por la falta de la misma en la lepra lepromatosa, que puede reconocer otra explicación, existen pocas pruebas de que la reactividad guarde la menor relación con la infección por el *M. leprae*. Se necesita confirmación de la opinión aceptada habitualmente de que los enfermos de lepra tuberculoidea son positivos casi universalmente. Deben hacerse comparaciones con hermanos sanos, equiparando cuidadosamente los grupos con respecto a edad y sexo. Hay que buscar además datos relativos a la teoría de que la creciente frecuencia de la reactividad con la edad es puramente un fenómeno de "maduración." Podrían obtenerse los mismos comprobando, en varias regiones, grupos de niños que discrepen con respeto a condiciones estipuladas del ambiente, pero comparables en otros sentidos.

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