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Immunological effects of lepromin testing in Sri Lankan population groups. I. Effect of repeated lepromin testing

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Summary It has been reported that lepromin testing in human subjects induces sensitization, and that with repeated testing the incidence of 'positive' Mitsuda reactions increases. On repeated testing in two Sri Lankan population groups, with Mitsuda reactions of 6 mm or less, we found that a second lepromin test at 28 days seemed to induce tolerance with reduction in reaction size or even zero reactions. This tolerance phenomenon was seen markedly with Mitsuda reactivity and less so with Fernandez reactivity. There was evidence also that while tolerance seemed to be occurring with the second test, a third test at 56 days seemed to reinduce and elicit resensitization, though weakly, with both types of reactivity. Evidence is also produced that reactors and non-reactors with both Fernandez and Mitsuda reactivity, behave differently on repeated lepromin testing, suggesting that immunologically they are different population groups.

Introduction

It is now believed that the Mitsuda response observed with the lepromin test is a 'vaccination' response,[1] and that lepromin testing by itself may induce sensitization to a subsequent lepromin test. Thus it has been shown that repeated testing with lepromins of human origin[2] and also armadillo origin[3] would increase the incidence of 'positives' in the population so tested; Dharmendra and Chatterji[2] also showed that the incidence of leprosy, in those tested repeatedly—but yielding a persistently negative response on examination 16 years later, was markedly high. Further, a recent report from Cuba,[4] found that lepromin testing with lepromins of human origin, induced antibody formation detected by the FLA–ABS test persisting up to 180 days after lepromin testing. Thus the presently

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available evidence strongly supports the view that lepromin testing induces immune responses, and that these may be of both the humoral- and cell-mediated types.

The study reported here is of an investigation of the pattern of sensitization occurring with repeated lepromin testing in Sri Lanka.

Materials and methods

The methods of skin testing and reading of results have been described in detail elsewhere.[5] The protocol followed for repeat testing was as described by the Third IMMLEP Scientific Working Group.[6] Briefly the methodology was as follows.

Two population groups in Sri Lanka were investigated (at Pussellawa and Pedro). Their characteristics and pattern of reactivity on initial lepromin testing have been described earlier.[5] After initial lepromin testing (using lepromin A with a bacillary content of 3×10^7 bacilli/ml)—after which both Fernandez and Mitsuda reactions were read; those who had a Mitsuda reaction diameter of 6 mm or less, were similarly retested on an upper volar site on the opposite arm. Following the second retest Fernandez and Mitsuda reactivity were read again. At Pussellawa, where the study was carried out first, a third retest was carried out at a lower volar site of the forearm (with a single test),* when the second Mitsuda reaction was read, the plan being, again, to retest only those showing reactions of 6 mm or less. However, only three individuals showed such increases in reaction size, and therefore with the exclusion of these individuals all the others were retested a third time. At Pedro (where testing was done after Pussellawa), only one retest with lepromin A (a second test) was carried out. On each occasion of a test (or retest), both Fernandez and Mitsuda reactivity were read.

The number of individuals in whom the results of such repeated retesting were available is listed in Table 1.

Table 1. Availability of results (number of persons) with lepromin retest results (including 'soft' reactions)

	First retest results (second test)			Second retest results (third test)			Where both first and second retest results are available		
Area	Fernandez	Mitsuda	Both	Fernandez	Mitsuda	Both	Fernandez	Mitsuda	Both
Pussellawa	101	137	94	73	80	63	58	80	50
Pedro	65	67	35						

* A tuberculin test had been done on the same forearm with the first lepromin test.

Results

In Figure 1 are presented the frequency distributions of Mitsuda reactivity to lepromin A in the two areas on first testing. Figures 2 and 3 show the frequency distributions of Mitsuda reactions elicited on retesting of those showing reactions of 6 mm or less with the initial test.

Figure 4 shows the frequency distributions of Fernandez reactivity to lepromin A. Figures 5 and 6 show the frequency distributions of Fernandez reactions of those retested, whose initial Mitsuda reaction size was 6 mm or less.

The change observed here with Mitsuda reactions on retesting the first time (second lepromin test), is one of reduction in size, demonstrating the induction of 'tolerance'. In fact many reactions of larger sizes ('reactors' or 'positives' of 3 mm or more)[5] became totally negative (0 mm) (43% of the whole at Pussellawa and 25% at Pedro). Further, only approximately 10% of 'non-reactors' or 'negative' Mitsuda reactors of 2 mm or less, became 'reactors' (positive) with the first retest. Of the whole retested population in both areas only approximately 4% showed a 'significant'* increase (of more than 2 mm over the first test) in reaction size. On the other hand, approximately 60% at Pussellawa and 44% at Pedro showed 'significant' reductions in Mitsuda reaction size with the second test.

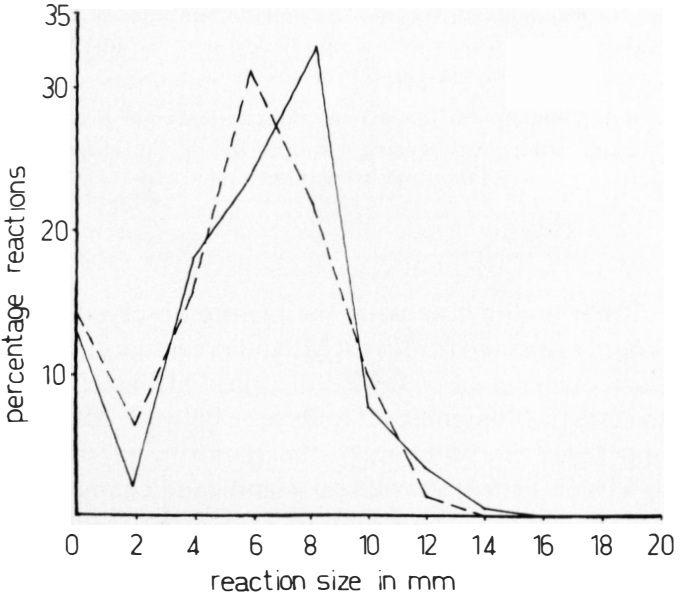

Figure 1. Frequency distributions of Mitsuda reactions to lepromin A with the first test at Pussellawa ( ) and Pedro (– – – – –).

* It should be noted that 'significant' (when within quotes) refers to a change in reaction size (an increase or decrease) of more than 2 mm making allowance for possible vagaries of testing and reading procedures and does not mean significant in the statistical sense.

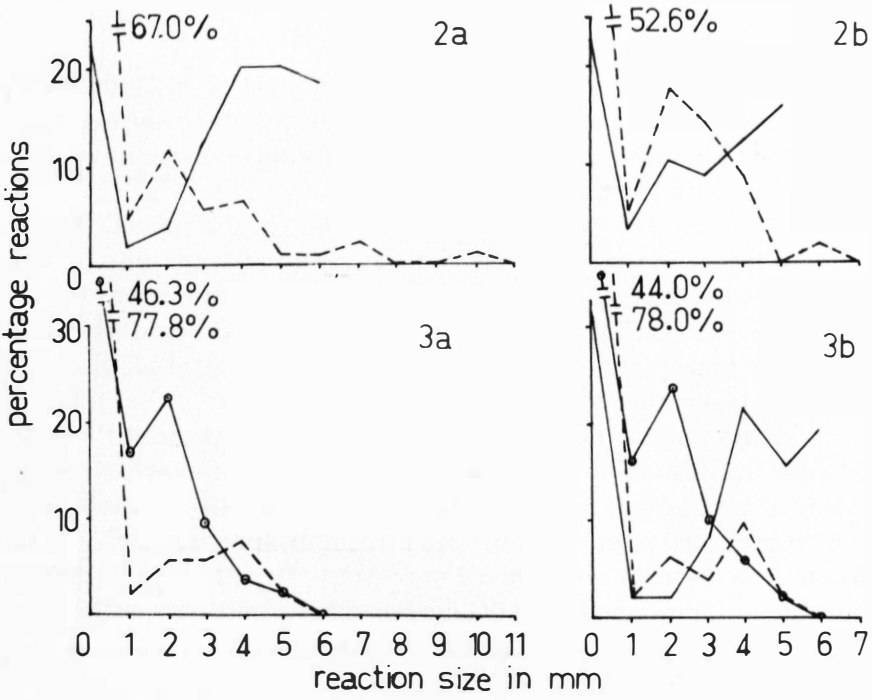

Figure 2. Frequency distributions of Mitsuda reactions to lepromin A of the same individuals, whose first test Mitsuda reaction sizes were 6 mm or less with the first (————) and second (– – – – – –) tests (a) at Pussellawa, and (b) at Pedro.

Figure 3. Frequency distributions of Mitsuda reactions to lepromin A of the same individuals, whose first test Mitsuda reaction sizes were 6 mm or less at Pussellawa (a) with the second (– – – – – –) and third (——o——) tests and (b) with the first (————), second (– – – – – –) and third (——o——) tests.

In the case of Fernandez reactivity, the picture observed with the frequency distributions is not as distinctive as with Mitsuda reactivity. While the frequency distributions show evidence of possible 'tolerance' at Pussellawa, the pattern at Pedro shows no statistically significant difference between the distributions of the first and second tests. In both areas the majority of individuals (52% at Pussellawa and 67% at Pedro) showed no 'significant' change in reaction size. A small number (17% at Pussellawa and 14% at Pedro) showed a 'significant' increase in reaction size, while reduction in reaction size was shown by 31% at Pussellawa and 19% at Pedro.

With the third lepromin test, which was carried out only at Pussellawa, both Mitsuda and Fernandez reactivity seem to be showing patterns suggestive of the recurrence or increase of reactivity. With Mitsuda reactivity the increase in reaction sizes (between the second and third tests) is of the smaller sizes in the 1– to 3-mm range, and therefore (allowing for a 2-mm variability in size), roughly the

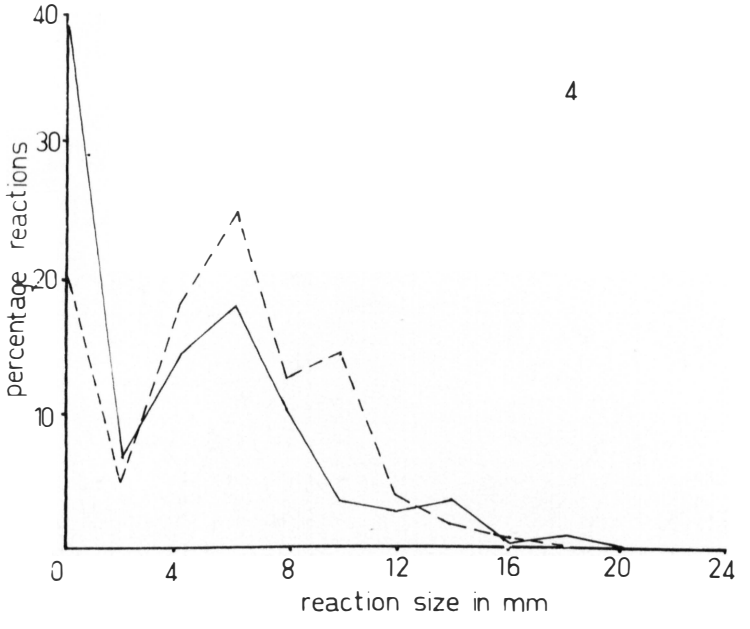

Figure 4. Frequency distributions of Fernandez reactions to lepromin A with the first test at Pussellawa (————) and Pedro (– – – – – –).

same small numbers of individuals (7 and 5% respectively) showed increases or decreases of reaction sizes. While the majority (54%) showed no change with Fernandez reactivity, 35% showed 'significant' increases of reaction size with 11% showing a reduction.

Comparing similarly the first and third lepromin tests, with Mitsuda reactivity, 9% showed an increase and 44% a decrease, with 47% no change. With Fernandez reactivity the numbers were 27%, 13% and 60% respectively.

The correlations between the first, second and third lepromin tests were statistically evaluated using the technique of regression analysis and nonparametric statistical methods. These analyses added further detail to the gross patterns observed in the frequency distributions. The results of the above analyses may be summarized as follows:

A *With Mitsuda reactivity*

1 There was a significant difference of the trend in the change of reactivity between the first and second tests, in the reactors ($\geqslant 3$ mm) as compared with the non-reactors ($\leqslant 2$ mm)[5].

2 The reactors showed a reduction in reactivity between the first and second tests, while the non-reactors showed no change.

3 In the subsets where the numbers of results available permitted statistical

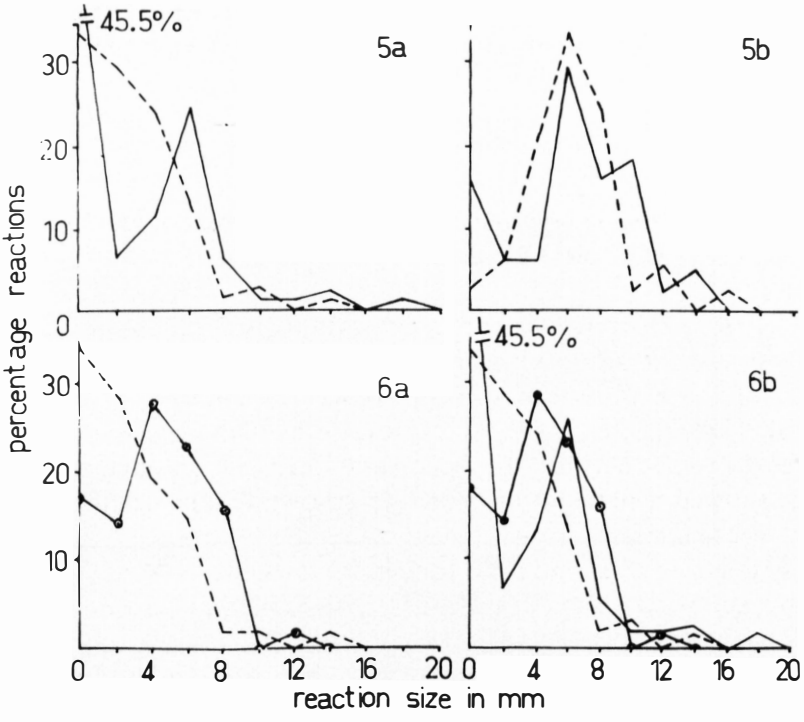

Figure 5. Frequency distributions of Fernandez reactions to lepromin A of the same individuals, whose first Mitsuda reaction sizes were 6 mm or less with first (————) and second (– – – – –) tests at (a) Pussellawa and (b) Pedro.

Figure 6. Frequency distributions of Fernandez reactions to lepromin A of the same individuals, whose first Mitsuda reaction sizes were 6 mm or less at Pussellawa, with (a) the second (– – – – –) and third (——o——) tests and (b) the first (————), second (– – – – –) and third (——o——) tests.

Table 2. Occurrence of 'soft' Mitsuda reactions with repeated lepromin testing. (Total number tested and read (including 'soft' reactions) within parenthesis)

Area	With first test	With second test	With third test
Pussellawa	1%	17%	15%
	(241)	(137)	(80)
Pedro	4%	18%	
	(161)	(67)	

analysis, the difference between the second and third tests also, was that reactors in the second test showed a reduction in reaction size, while the non-reactors showed an increase in reaction size.

4 Comparison of first and third tests also showed similar patterns with an increase in reaction sizes of reactors and diminution of reaction sizes of non-reactors.

B *With Fernandez reactivity*

Again, as with Mitsuda reactivity, the trend of changes of reactivity between the first and subsequent tests was different between the reactors ($\geqslant 3$ mm) and non-reactors ($\leqslant 2$ mm).[5]

2 The difference observed between the first and the second test was a reduction in reactivity in the reactor group and an increase in reactivity in the non-reactor group (a trend different from that in the Mitsuda reaction).

3 In subsets where the numbers of results available permitted statistical analysis, the difference between the second and third tests was that reactors in the second test showed a reduction in reaction size while non-reactors showed an increase in reaction size.

4 Comparison of the first and third Fernandez tests also showed that there were increases in reaction sizes of the non-reactors and reduction in reaction sizes in the reactors.

All the above discussed changes (with both Fernandez and Mitsuda reactivity) were significant at an $\alpha \geqslant 0{\cdot}01$ level. The analysis also showed that the changes found with Mitsuda reactivity were not in any way influenced by sex, geographic location or BCG vaccination status. With Fernandez reactivity too, sex and BCG vaccination status seemed to have no influence on the results, whereas there is a possibility that geographic location does.

The relationship between the first and second, second and third, and first and third lepromin tests (in so far as Fernandez and Mitsuda reactivity are concerned) could be further examined by comparing the conversion of reactors to non-reactor status and vice versa with each subsequent test using McNemar's nonparametric statistical test for related samples.[7] With this evaluation it was found that with Mitsuda reactivity in both areas with the first retest there was a significant incidence of those changing from reactor to non-reactor status ($\alpha \geqslant 0{\cdot}01$) as compared with those showing a change from 'non-reactor' to 'reactor' status. There was no difference between the second and third tests at Pussellawa in this respect, while the first and third tests showed a similar difference to that between the first and the second. In other words the change seen between the first and second tests seemed to persist to the third test as well. With Fernandez reactivity the situation was different. There was no statistical difference in the changes of reactor/non-reactor status between first and second tests, whereas there was a significant ($\alpha \geqslant 0{\cdot}01$) increase in the incidence of reactors between the second and third tests and also the first and third tests. In other words, Fernandez reactivity shows an immunizing effect between first and third tests and second and third tests; thus while the first lepromin retest (second

test) showed no changes from the first, with the third test there was an increase in size (at Pusellawa). Thus the increase in reactivity (of both Fernandez and Mitsuda types) with the third test seen in the pattern of the frequency distributions at Pussellawa, is due to 0-mm reactions in the second test, becoming 1- or 2-mm reactions in the third test.

The foregoing analysis examines trends with repeated testing of the whole population group. At Pussellawa where three lepromin tests were carried out, the patterns of reactivity observed in each individual, with the three tests, could also be examined. Hence an individual could, in any one test, be a reactor or non-reactor, and any individual, therefore, could show any one of eight patterns of reactivity, ranging from reactor in all three tests to non-reactor in all three tests. The patterns of reactivity thus observed, showed the induced tolerance with Mitsuda reactivity clearly; and also revealed the difference between Mitsuda and Fernandez reactivity patterns with repeated testing (statistically significant at $\alpha \geqslant 0{\cdot}01$).

The above analysis of individual reaction patterns would also allow an opportunity of comparing the similarities and differences between Mitsuda and Fernandez reactivity patterns of individuals. The comparison here, showed that in any one individual, no discernible trend or correlation between Mitsuda and Fernandez reactivity patterns in the three tests could be made.

In the analysis described above only 'typical' Mitsuda reactions have been included (a well circumscribed and defined nodule was defined as 'typical'). However, some Mitsuda reactions did not manifest as above, and had a soft and sometimes plaque-like character. The significance of the latter is uncertain.[5] The occurrence of 'soft' Mitsuda reactions with the different tests is presented in Table 2. It is seen that there is a marked increase in such reactions with repeated testing.

Discussion

The results of this study are at variance with those of studies reported earlier[2,3] which described only sensitization, or persistent non-reactivity, with repeated lepromin testing, and make no mention of the possibility of the induction of tolerance. The tolerance described here may be possibly of a transient nature but was nevertheless shown by many of those tested. Lepromin consists of killed whole *Mycobacterium leprae*, and hence this tolerance response between the 28th and 56th day (and perhaps beyond) is to the latter. It is tempting to speculate whether such tolerance could occur in the early days of natural infection with viable *M. leprae* too.

Tolerance with mycobacterial infection is a well-known phenomenon. The best-known example of this is with *M. leprae* in lepromatous leprosy. The induction of tolerance with *M. leprae* has also been demonstrated in experimental situations, where in the mouse the intraperitoneal and intravenous routes of

administration lead to tolerance, while the intradermal route leads to sensitization.[8] In the investigation reported here the intradermal route in man seemed to induce at least a transient tolerance in contrast to the finding in mice.

A distinctive finding in this study was the different behaviour of individuals of the reactor and non-reactor categories (with both Fernandez and Mitsuda reactions) on repeated lepromin testing. These two groups seem to be showing a different immunological responsiveness, and perhaps belong to two different populations. The results here also appear to validate the conclusions drawn earlier as to the points at which separation of reactor and non-reactor should be made.[5]

It was found here, regarding the change of reactor/non-reactor status, that Fernandez reactivity and Mitsuda reactivity seemed to behave differently with repeated lepromin testing. Further, the results with the initial test and with the repeated tests seemed to show differing correlations with tuberculin sensitivity with the two types of lepromin reactivity. Both lepromin reactions, though evoked by suspensions of killed, whole *M. leprae* are said to be aetiologically different.[9,10] Fernandez reactivity is considered analogous to the tuberculin response[11,12] in *M. tuberculosis* infection; and the Mitsuda reaction, to BCG vaccination, in that lepromin itself induces reactivity to itself as does the latter,[13,14] namely a 'vaccination response.'[1]

One possible explanation for the differences may be that Fernandez and Mitsuda reactivity are elicited by different antigens of *M. leprae*. Convit *et al.*[15] have shown that if the bacteria free supernatant of lepromin was used in skin tests, it produces a reactivity identical to the Fernandez reaction of whole lepromin. On the other hand Mitsuda type reactivity would be induced only by whole bacteria. If this hypothesis (of differences of antigens) be true, then antigens which manifest with Fernandez reactivity do not seem to recognize 'tolerance' as clearly, or at the same level, that is identified with Mitsuda reactivity. Also Fernandez reactivity in eliciting reactivity analogous to that of the tuberculin type, would only recognize pre-existing hypersensitivity of the latter type, and play no role in inducing the latter.

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