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Immunological effects of lepromin testing in Sri Lankan population groups. II. Effect on reactivity to a soluble protein antigen of *Mycobacterium leprae*

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Summary Reactivity to the Soluble Protein Antigen (SPA) of *Mycobacterium leprae* is altered in a small but measurable way following the lepromin test when the retest with SPA is done one month after the lepromin test. The alteration resembles that shown by Fernandez reactivity in Sri Lanka after repeated lepromin testing, with nonreactors showing increases and reactors showing decreases of reaction size. The change observed is seen in greater numbers in the reactors. Sex and BCG vaccination status does not seem to effect the result of the post-lepromin SPA test. Of the three reactivities to antigens of *M. leprae* (SPA, Fernandez and Mitsuda), the factor that seems to influence the behaviour of post-lepromin SPA reactivity most is the reactivity status (reactor/nonreactor) of the SPA pre-lepromin test.

Introduction

If lepromin acts as a vaccine,[1] it could be assumed that following lepromin testing, reactivity to the Soluble Protein Antigen (SPA) of *Mycobacterium leprae* would be increased, both in the incidence of reactions and in size of reactions. We report here an investigation of the effect of lepromin testing on reactivity to SPA, in two Sri Lankan population groups.

Materials and methods

The populations tested were selected for expected differences in levels of nonspecific mycobacterial sensitization (low at Mahagastota, and high at Galagedera). The characteristics of these populations and the test methodology have been described elsewhere.[2,3] However, in this study, in addition to the first

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test with SPA, done concurrently with the lepromin test, when reading Mitsuda reactivity (on the 28th day after the test), a second test with SPA was carried out (on the volar aspect of the opposite forearm), which in turn was read at 72 h.

The results of such repeated testing were available in 153 BCG unvaccinated and 119 BCG vaccinated (total 272) individuals, in both areas, for analysis.

Results

In Figures 1 and 2 are presented the frequency distributions of SPA reactions elicited with the first and second (post-lepromin) tests in the two areas.

If individual reactions to SPA on the initial and retest be examined, the reactions may be expected to show either an increase or decrease, or no change in reaction size with the post-lepromin retest. If allowance be made for the vagaries of test and reading procedure and a change of up to 20 mm be allowed to account for such change, then it is found that the majority showed no change in reaction size (as defined above) (64% at Mahagastota and 54% at Galagedera respectively), while 24% showed an increase and 12% a decrease at Mahagastota, and 23% showed an increase and another 23% a decrease at Galagedera. Thus considerable numbers of individuals do show some change on the post-lepromin repeat test.

In the earlier report on the effects of repeated lepromin testing, it was shown that with the two types of reactivity with the lepromin test (Fernandez and Mitsuda), the nonreactor and reactor groups behave differently.[4]

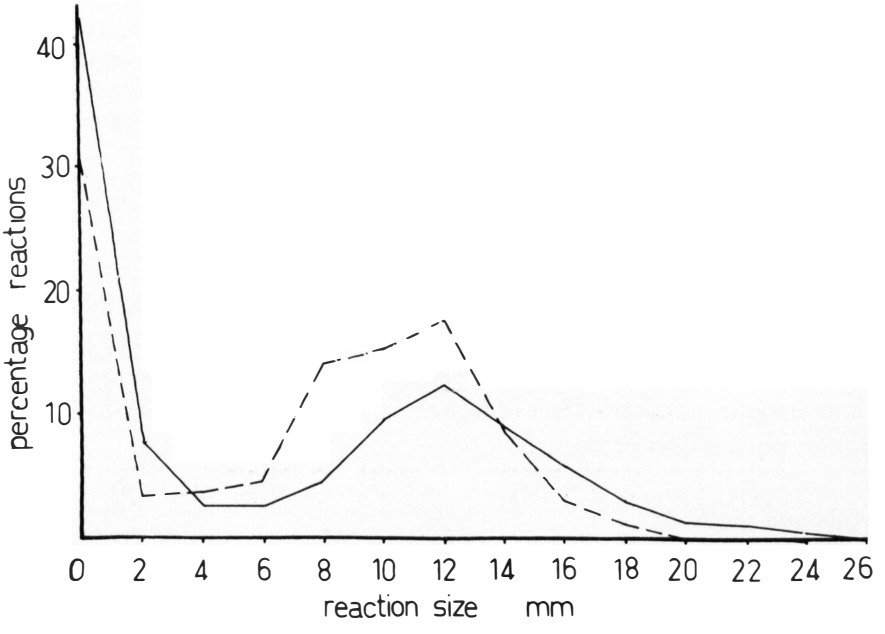

Figure 1. Frequency distributions of reactions to SPA with the first test at Mahagastota (——) and Galagedera (– – – –).

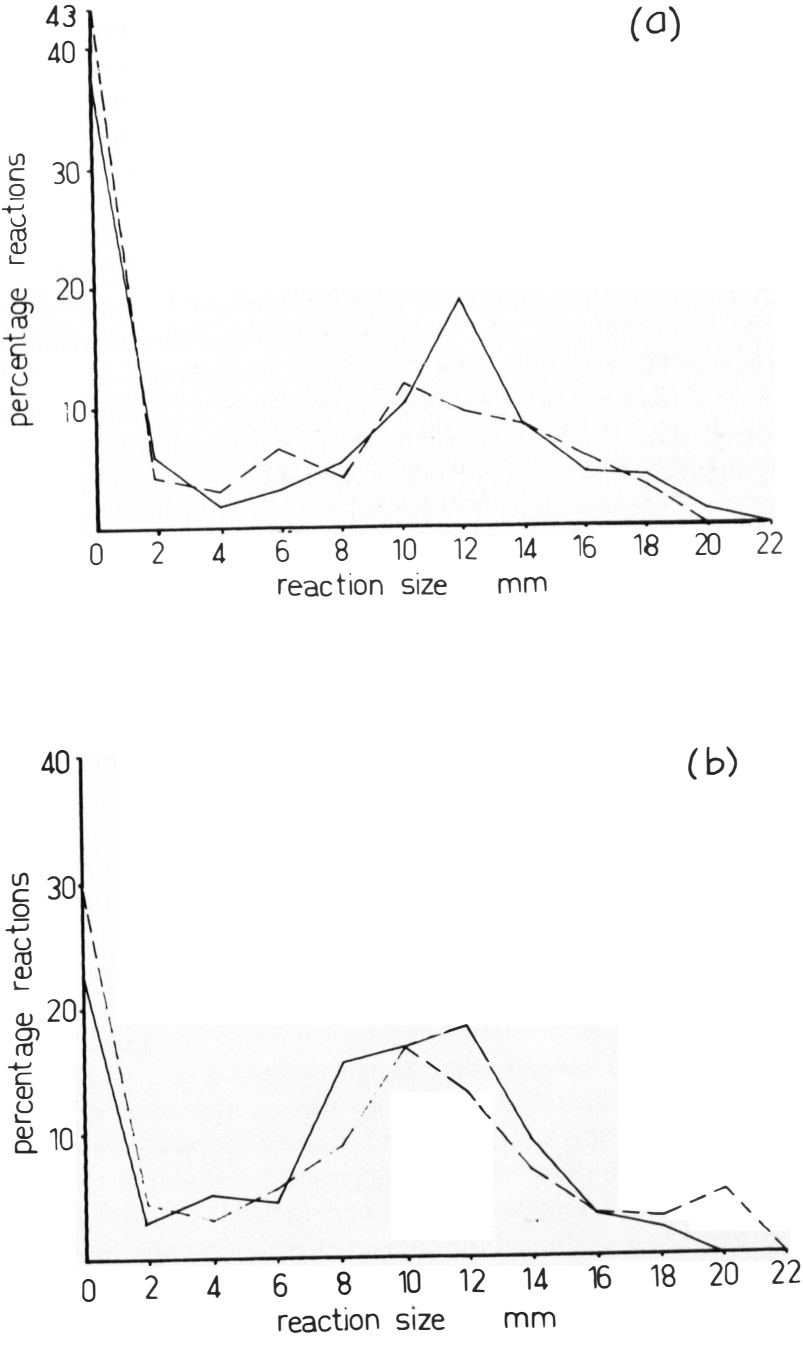

Figure 2.(a) Frequency distributions of reactions to SPA with the first test (——) and second test (– – – –) at Mahagastota. (b) Frequency distributions of reactions to SPA with the first test (——) and second test (– – – –) at Galagedera.

Hence, the behaviour with the retest, of the nonreactor (reaction size ≤ 4 mm) and reactor (reaction size ≥ 5 mm) groups[3] with the initial SPA tests were examined, (including for analysis any changes of reaction size of even 1 mm). Examination of the whole group in the two localities showed that while considerable numbers showed no change, a larger number of reactors in both areas showed reduction in reaction size (as compared with those showing an increase); while among nonreactors, a larger number showed an increase in reaction size as compared with those showing a reduction. Statistical evaluation of the change (using McNemar's nonparametric test for unrelated samples)[5] showed that the changes observed at Mahagastota in both reactors and nonreactors were significant at a 1% level, while at Galagedera the change in reactors was significant only at a 10% level and in nonreactors at a 5% level.

Further examination of this data with relevance to sex and BCG vaccination status, showed that the post-lepromin test SPA reaction changes were not statistically significant in any subgroup at Galagedera, except in one (of four) — at a 5% level, while at Mahagastota all subgroups showed changes significant at a 5% level. The change of immunological status (nonreactor to reactor and vice versa) with SPA reactivity following the lepromin test was also examined with reference to the latter variables. Of the subgroups examined, again no statistically significant changes were found, except in one at Galagedera (significant at a 5% level).

The fate of the SPA retest in relation to immunological status, as determined by Fernandez and Mitsuda reactivity with the lepromin test, was also examined (Table 1), (the increase or decrease of SPA reactivity considered was of any amount — 1 mm or more). It is seen that, while with the Fernandez test, the nonreactor group showed equivocal results in the comparison of those showing increases, with those showing decreases of reaction size, with both Fernandez reactors and Mitsuda nonreactors and reactors, there were larger numbers showing decreases than those showing increases. Again with both Fernandez and Mitsuda reactivity, those showing no change of reaction size were predominantly of the nonreactor groups, while the reactors showed more changes (of either increase or decrease). However, the changes were not statistically significant. Similar analysis of separately matched groups on the basis of geographical location, BCG vaccination status, sex and nonreactor/reactor status showed a significant difference (in those showing increases/decreases) in only one group, namely BCG vaccinated, female, Mitsuda nonreactors at Galagedera ($\alpha \geq 0{\cdot}05$).

The above analysis examined the change of post-lepromin SPA reactivity in relation to Fernandez and Mitsuda reactivity status, in the whole population. Next, was examined, the changes seen in the post-lepromin SPA test in the nonreactor and reactor groups in relation to Fernandez and Mitsuda reactivity status, also considering the SPA — first test status, i.e. whether a nonreactor or reactor in the latter. The conclusions of the above analysis were that, irrespective of Fernandez and Mitsuda reactivity status (whether reactor/nonreactor):

Table 1. Fate of the post-lepromin SPA test as compared with the first SPA test in relation to Fernandez and Mitsuda reactivity

Fate of SPA retest as compared with first test		Percentage individuals			
		Fernandez		Mitsuda	
		Nonreactor	Reactor	Nonreactor	Reactor
Reaction size increased	Mahagastota	13·5	15·3	6·3	22·5
	Galagedera	7·2	26·6	6·4	30·4
	Total	9·6	21·6	6·3	26·5
Reaction size decreased	Mahagastota	11·7	27·9	8·1	30·6
	Galagedera	8·8	36·8	9·6	34·4
	Total	9·6	30·8	8·4	32·3
Reaction size shows no change	Mahagastota	27·0	5·4	17·1	15·3
	Galagedera	11·2	11·2	10·4	12·0
	Total	17·6	8·0	13·4	13·4

1 Nonreactors in the SPA first test, showed, with the retest, in all groups, a persistently higher proportion of those showing increased reactivity as compared with those showing a reduction; however these differences in all subsets, were not statistically significant, except in the Fernandez nonreactors ($\alpha \geqslant 0{\cdot}05$).
2 Reactors in the SPA first test showed an increase in those showing a reduction in reactivity (as compared with those showing an increase). However, of the two geographical locations, only the population subsets at Mahagastota showed differences which were statistically significant ($\alpha \geqslant 0{\cdot}05$), while at the other location at Galagedera, though the trend was similar, no subsets showed statistically significant differences.

Thus, when the three variables of the immunological status, *vis à vis*, the nonreactor and reactor of SPA first test, or Fernandez or Mitsuda reactivity were considered together, for their effect on the SPA retest, the results of the latter varies according to the status of the SPA first test, irrespective of Fernandez and Mitsuda reactivity.

Discussion

The lepromin test effects the immunological status of the individual as evaluated using the SPA test in a small but measurable and significant way. The change seen in the post-lepromin SPA retest is not as striking as that shown by the reactor group in Mitsuda reactivity of the lepromin test with repeated lepromin testing.[4] The trend of the changes with the SPA retest, with nonreactors with the first SPA

test tending to show increases, and reactors showing decreases of reaction size, though, is similar to that showed by Fernandez reactivity with repeated lepromin testing.[4] The strength of these changes is weak, in that unlike with Mitsuda reactivity, the change does not alter the immunological status of the individual whether a nonreactor or reactor.

It is noteworthy that with all three reactivities to antigens of *M. leprae* (Fernandez, Mitsuda and SPA), the trend of any change, induced by the post-lepromin retest is similar in relation to pre-lepromin test immunological status, whether reactor or nonreactor with each. However, the effect of the status of each of the two former reactivities on SPA retest reactivity seem of lesser consequence, than that of the SPA first test status itself. This brings to mind the question of the correlation of the various types of reactivity with each other. While there is literature on the correlation between Fernandez and Mitsuda reactivity,[6,7] there seems to be a dearth of that between SPA and Fernandez, and SPA and Mitsuda reactivity.

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