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Experiences with *Mycobacterium leprae* soluble antigens in a leprosy endemic population

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Summary Rees and Convit antigens prepared from armadillo-derived *Mycobacterium leprae* were used for skin testing in two leprosy endemic villages to understand their use in the epidemiology of leprosy. In all, 2602 individuals comprising 202 patients with leprosy detected in a prevalence survey, 476 household contacts and 1924 persons residing in non-case households were tested with two antigens. There was a strong and positive correlation ($r = 0{\cdot}85$) between reactions to the Rees and Convit antigens. The distribution of reactions was bimodal and considering reactions of 12 mm or more as 'positive', the positivity rate steeply increased with the increase in age. However, the distributions of reactions to these antigens in patients with leprosy, their household contacts and persons living in non-case households were very similar.

These results indicate that Rees and Convit antigens are not useful in the identification of *M. leprae* infection or in the confirmation of leprosy diagnosis in a leprosy endemic population with a high prevalence of nonspecific sensitivity.

Introduction

There are several unanswered questions in the epidemiology of leprosy. There has been a long-felt need for a test that could recognize infection with *Mycobacterium leprae* and thereby serve as a marker for the postinfection phase.[1] Lepromin testing does not fulfil this need because studies[2] have shown lepromin positivity in leprosy non-endemic populations, as well as its occurrence on account of other mycobacterial infections. Also, it is widely believed that the late lepromin reaction (Mitsuda reaction), which serves as a tool to classify patients with leprosy,[3] helps to identify persons susceptible to the lepromatous form of leprosy.[4,5] Reaction to lepromin is expected to be negative in such persons, although infected with *M. leprae*. In addition, lepromin has been documented as a miniature vaccine.[6] Drs Rees and Convit have prepared soluble antigens from *M. leprae* of armadillo origin, known as Rees and Convit antigens, which have been used in different parts of the world to understand their use in the epidemiology of leprosy.[7] Rees

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and Convit antigens were supplied to our Unit by the IMMLEP programme of the World Health Organization. After obtaining the necessary clearance from the Drugs Controller of India, we used these antigens for skin testing in a leprosy endemic population, and our findings are reported here.

This study was particularly relevant to us as our Unit is proposing to undertake a leprosy vaccine trial. Identification of *M. leprae* infected population and also the population at risk of suffering from leprosy, particularly the multibacillary form, will be highly relevant in a leprosy vaccine trial situation.

Materials and methods

This work was done during the period February to April 1987, in a population of 2602 persons from two leprosy endemic villages in Sriperumbudhur Taluk of Chengalpattu District in Tamil Nadu. Initial screening for leprosy prevalence in this population was done by trained paramedical workers. All the suspects and cases of leprosy detected by the paramedical workers were reexamined by a medical officer or one of two senior technical assistants, who had more than 15 years experience and had been trained in standardized clinical diagnosis of leprosy. Diagnosis of leprosy was based on clinical parameters and was supported by skin-smear examination for acid-fast bacilli. Patients were classified into different types following the system adopted by the Indian Association of Leprologists.[8] In Chengalpattu District, BCG immunization was not undertaken by the health services, as this district was kept free from BCG coverage. However, each person was examined by a trained technician for the presence or absence of a BCG scar and the result recorded.

In the various observations reported here, the following antigens were used for skin tests:

(1) Tuberculin PPD (RT 23, 1 TU per dose) from BCG Vaccine Laboratory, Madras.
(2) Rees antigen (Batch CD-19), supplied by Dr Rees (1 mcg protein per dose).
(3) Convit antigen (Batch SA-IND, 1.16.86) supplied by Dr Convit (dose as standardized by Dr Convit).

For skin testing and reading standard procedures were followed.[9] Skin testing was done by an assessed tester by superficial intracutaneous injections of 0·1 ml of antigens. Tuberculin test was given on the mid-volar side of the left forearm. Rees and Convit antigens were randomly allocated to the upper dorsum of the two forearms. Reactions to Rees and Convit tests were read after 48 hr and that to the tuberculin test after 72 hr. The interval of 48 hr for Rees and Convit antigen readings was based on our previous experience, where we took serial readings after 24, 48 and 72 hr.[10] Tuberculin skin tests are routinely read after 72 hr.[9] Transverse diameters of the indurations were measured in millimetres, by an experienced reader. The same tester and reader were used throughout the study. Skin testing was done in all age and sex groups, excluding infants up to the age of 1 year.

After initial standardization studies for skin testing,[10] about 2400 individuals from one village (TT01) were tuberculin tested and read. Three months later, Rees and Convit antigen skin tests were performed in the same population. Data from 888 individuals who were tested and read for all the three skin test antigens, tuberculin, Rees and Convit, has

been used to compare the skin test reactions to these three antigens. In this village 126 individuals were tested and read for the Rees and the Convit antigens, but not for tuberculin.

Skin testing was performed using Rees and Convit antigens in an adjoining village (TT02). Tuberculin was not used in this village.

The skin test responses to the Rees and the Convit antigens were studied in the population from both the villages with respect to age, sex, clinical evidence and type of leprosy, as well as according to the household contact status with leprosy patients. From village TT01, 96 patients, 143 household contacts of these patients and 812 individuals living in households without a case of leprosy were test read. From village TT02, these figures were 106, 333 and 1112 respectively. In all, 2602 individuals, comprising 202 clinically diagnosed patients with leprosy during the prevalence survey, 476 household contacts of these patients and 1924 individuals living in households without a case of leprosy were tested and read for the Rees and Convit antigens. Of the 476 household contacts, 444 individuals were contacts of paucibacillary cases and 32 were contacts of multibacillary cases.

Results

COMPARISON OF REACTIONS TO REES AND CONVIT ANTIGENS AND TUBERCULIN

Correlation between reactions to Rees and Convit antigens in 888 individuals from village TT01 is presented in Table 1. There was a strong and positive correlation between the two indurations with a correlation coefficient of 0·85 which was statistically highly significant ($p < 0{\cdot}001$). However, the mean size of reaction to Rees antigen (14·1 mm) was slightly higher than that to the Convit antigen (12·8 mm). The mean difference between the two reaction sizes was 1·2 mm ($p < 0{\cdot}001$). This relationship was similar in different age and sex groups as well as in patients and their household contacts. The relationship was also similar in the 1551 individuals from village TT02 (correlation coefficient 0·80; $p < 0{\cdot}001$).

Similar comparisons were made between reactions to Rees antigen and tuberculin, as well as between reactions to Convit antigen and tuberculin in the 888 individuals from

Table 1. Correlation between reactions to Rees and Convit antigens in 888 individuals from village TT01

Reaction to Rees antigen	Reaction to Convit antigen (mm)							
	0–3	4–7	8–11	12–15	16–19	20–23	24–27	Total
00–03	14	4	5	—	1	—	—	24
04–07	11	140	18	3	1	1	—	174
08–11	1	35	46	18	4	1	—	105
12–15	3	14	35	77	23	5	—	157
16–19	2	5	11	75	126	13	1	233
20–23	—	—	2	17	91	35	7	152
24–27	—	—	—	—	7	25	10	42
28–31	—	—	—	—	—	—	1	1
Total	31	198	117	190	253	80	19	888

village TT01 (Table 2). It was seen that there was a positive but weak correlation between reactions to Rees antigen and tuberculin (correlation coefficient 0·44; $p < 0·01$) as well as between reactions to Convit antigen and tuberculin (correlation coefficient 0·44; $p < 0·01$). The mean difference in sizes of reaction to Rees antigen and tuberculin was 2·7 mm ($p < 0·001$) and that between Convit antigen and tuberculin was 1·5 mm ($p < 0·001$).

Bimodality in the frequency distributions was seen for all the three antigens with the antimode at 8–11 mm (Figure 1). In analogy with tuberculin reactions, considering the first curve with reactions of 0–11 mm to represent 'reaction negative' individuals and the second curve with reactions of 12 mm and above to represent 'reaction positive' individuals, the proportions with 'positive' reactions to the Rees and the Convit antigens were 66% and 61% respectively. This difference was statistically significant ($p < 0·05$).

Comparison between reactions to the Rees antigen and tuberculin showed that, in general, Rees antigen positivity was higher in the tuberculin positive individuals. Patients of leprosy as well as individuals in whom BCG scars were present were excluded for the sake of this comparison. The results are based on skin test reactions in 702 individuals. It was seen that in the younger age group of 1–9 years, the Rees antigen positivity was much higher among tuberculin positives (66%) as compared to that among tuberculin negatives (24%). This difference was statistically highly significant ($p < 0·001$). However, in the higher age groups the differences were small and not statistically significant.

In the village TT01, 126 individuals who were not tested for tuberculin were tested and read for the Rees and Convit antigens. Distributions of reactions to the Rees and the Convit antigens in these individuals were very similar to the ones in the 888 tuberculin

Table 2. Correlation between reactions to Rees, Convit antigens and tuberculin in 888 individuals from village TT01

Reaction (mm) to	Reaction to tuberculin (mm)							
	0–3	4–7	8–11	12–15	16–19	20–23	24–27	Total
Rees antigen								
00–03	5	3	3	5	6	2	—	24
04–07	59	79	3	10	14	5	4	174
08–11	21	42	7	8	16	10	1	105
12–15	10	50	21	29	33	12	2	157
16–19	12	49	28	48	69	21	6	233
20–23	4	12	9	40	66	18	3	152
24–27	1	2	3	7	23	6	—	42
28–31	—	—	1	—	—	—	—	1
Total	112	237	75	147	227	74	16	888
Convit antigen								
00–03	4	6	4	5	8	4	—	31
04–07	68	87	4	12	21	2	4	198
08–11	14	54	10	10	17	11	1	117
12–15	13	52	24	41	38	21	1	190
16–19	9	31	25	60	95	23	10	253
20–23	4	6	7	16	36	11	—	80
24–27	—	1	1	3	12	2	—	19
Total	112	237	75	147	227	74	16	888

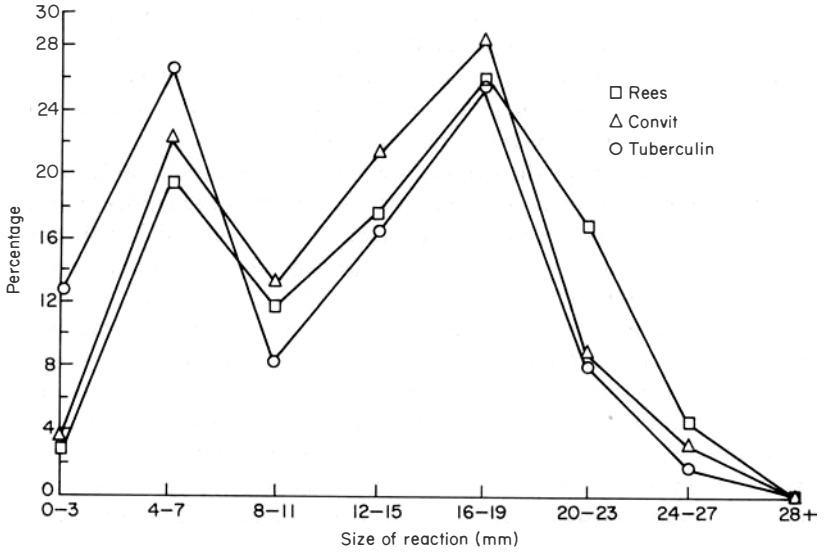

Figure 1. Distribution of the 888 persons by size of reaction to Rees and Convit antigens and tuberculin.

tested individuals. The mean reaction sizes for the Rees antigen were 14·3 mm and 14·1 mm and that for the Convit antigen 12·9 mm and 12·8 mm in the two groups of 126 and 888 individuals respectively. The proportion of individuals with positive reactions were also very similar in the two groups. 67·5% of the tuberculin tested and 65·9% of those not tested with tuberculin gave positive reactions to the Rees antigen. Similar figures for the Convit antigen were 62·7% and 61·0% ($p > 0{\cdot}5$, in both cases).

INFLUENCE OF BCG SCAR ON REES ANTIGEN POSITIVITY

The 2602 individuals from the two villages who were Rees antigen tested and read were also examined for the presence of a BCG scar. As mentioned earlier, this area was kept free from routine BCG vaccination and as such the prevalence of BCG scars was low. By the age of 10 years a high proportion of persons (68%) were showing Rees antigen positivity, and only children aged 1–9 years were considered in the study of the influence of BCG scars on the Rees antigen positivity. Of the 688 children aged 1–9 years who had been examined for BCG scars, 79 (11·5%) had BCG scars. Eighteen (22·8%) of the 79 with a BCG scar and 173 (28·4%) of the 609 without a BCG scar showed Rees positivity. This difference was not statistically significant ($p > 0{\cdot}2$).

It was also seen that the tuberculin positivity was similar in persons with a BCG scar and in those without. Considering the age group 1–9 years, 15 (11·2%) of 134 with a BCG scar and 54 (10·5%) of 515 without a BCG scar showed positive reactions to tuberculin ($p > 0{\cdot}9$).

REES SKIN TEST POSITIVITY IN PATIENTS AND NON-PATIENTS OF LEPROSY

Skin test results from the two villages with respect to the positivity to the Rees antigen was marginally different. It was slightly higher in village TT01 as compared to that in village

TT02. This difference was consistent in different groups of individuals (Table 3). However, distributions of reactions to the Rees antigen in both the villages were similar and bimodal with the antimode at 8–11 mm. Therefore, the information from the 2602 individuals in the two villages is merged for studying the pattern of reactions to Rees' antigen. Considering 12 mm and above as the criterion for positivity, 11·9%, 41·8%, 68·0% and 76·9% were 'positive' to Rees antigen in the age groups 1–4, 5–9, 10–14 and 15+ years, respectively. Figure 2 provides the information on Rees skin test positivity in different age groups separately for the 202 patients with leprosy, 476 household contacts of these patients and 1924 individuals living in households without leprosy.

Of the 202 patients, only 14 (7%) had multibacillary forms of leprosy and thus only 32 of the 476 household contacts were contacts of patients with multibacillary leprosy. Fourteen (43·8%) of the 32 contacts with multibacillary leprosy and 270 (60·8%) of the 444 contacts of patients with paucibacillary leprosy were 'positive' to the Rees antigen. This difference was not statistically significant ($0{\cdot}10 > p > 0{\cdot}05$). The Rees antigen positivity was 22·2% (4 of 18) and 41·2% (84 of 204) in the age group 1–14 years and 71·4% (10 of 14) and 77·5% (186 of 240) in the age group 15+ years in the contacts of multibacillary and paucibacillary cases respectively. These differences were also not statistically significant ($p > 0{\cdot}1$).

Considering the 202 patients with leprosy, frequency distributions of Rees antigen skin indurations are given separately for the different types of leprosy in Table 4. The bimodality of the distribution is observed in patients with leprosy as well. This bimodality is also seen in patients with tuberculoid and the borderline tuberculoid types. Amongst 14 patients with borderline or lepromatous leprosy, 4 showed reactions of 12 mm or more though there was no tendency for bimodal distribution. Since the significance of smaller reactions to the Rees antigen may be different from that to tuberculin, while comparing the results of Rees' antigen test in patients and non-patients of leprosy, the entire range of reactions were considered. Thus, when the frequency distributions (age and sex

Table 3. Rees antigen positivity (% with 12+ mm) according to age in the two villages

		Age group (in years)						
Village	Population	1–4	5–9	10–14	15–24	25–34	35+	Total*
TT01	Patients	—	47·8	63·7	80·0	93·1	78·2	66·5
		(2)	(11)	(15)	(20)	(16)	(32)	(96)
	Contacts	12·7	50·0	61·8	78·4	100·0	78·6	69·1
		(23)	(24)	(16)	(27)	(20)	(33)	(143)
	Others	13·1	43·5	70·5	79·8	84·9	85·1	69·0
		(110)	(123)	(124)	(152)	(116)	(187)	(812)
TT02	Patients	—	50·1	87·3	70·7	74·3	67·0	61·2
		(4)	(10)	(11)	(24)	(18)	(39)	(106)
	Contacts	11·0	49·6	57·4	74·9	91·0	67·7	62·9
		(53)	(54)	(52)	(60)	(32)	(82)	(333)
	Others	11·8	35·6	67·9	73·1	76·1	74·8	61·9
		(136)	(149)	(140)	(226)	(147)	(314)	(1112)

Figures in parentheses give denominators.
* Age-sex standardized.

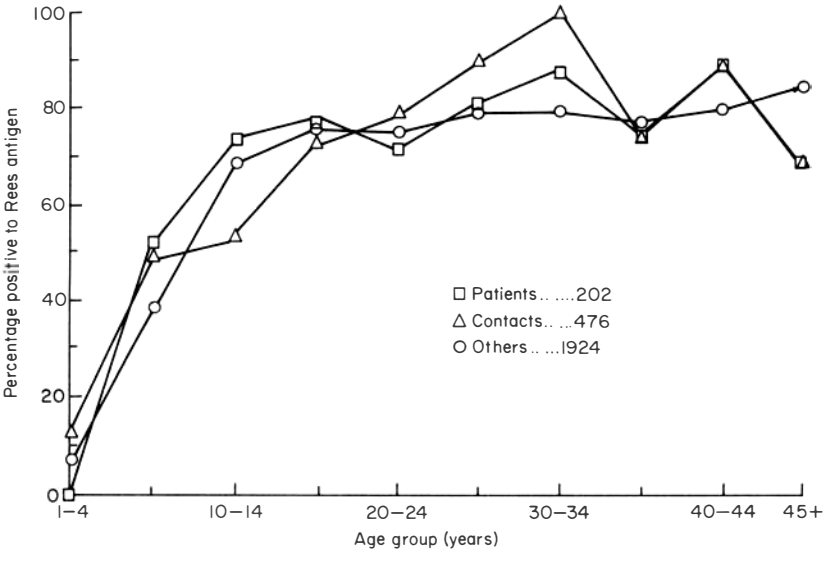

Figure 2. Rees skin test positivity (%) in patients, contacts and others by age.

Table 4. Distribution of leprosy patients by size of reaction to Rees' antigen

Type of leprosy	Size of reaction (mm)							
	0–3	4–7	8–11	12–15	16–19	20–23	24–27	Total
Neuritic	2	5	2	5	3	6	2	25
Indeterminate	1	8	3	13	8	5	3	41
TT and BT	3	16	10	30	32	19	12	122
BL and LL	3	3	4	2	1	1	0	14
Total	9	32	19	50	44	31	17	202

standardized) of Rees antigen indurations for the patients, contacts and the general population (excluding patients of leprosy and contacts) from the two villages were considered, the similarity in the distributions was very striking (Table 5). All the three groups gave a bimodal distribution with the antimode at 8–11 mm (Figure 3).

Discussion

Preparation of *M. leprae* soluble antigens was being viewed as a promising development to detect subclinical *M. leprae* infections. Studies in Venezuela by Convit indicated that the soluble antigen developed by him from the armadillo-derived *M. leprae* would meet the requirements and would be sufficiently sensitive and specific for epidemiological field studies.[11] IMMLEP–TDR under the World Health Organization encouraged studies using these antigens in several worldwide areas.[11]

Table 5. Proportion (%)* of patients and nonpatients of leprosy by size of reaction to Rees antigen

	Reaction to Rees (mm)								
Population	0–3	4–7	8–11	12–15	16–19	20–23	24–27	28–31	Total
Patients	4·0	23·9	8·7	22·3	20·5	13·2	7·4	—	100
Contacts	3·6	19·6	12·0	18·1	23·8	18·8	3·6	0·5	100
Others	4·2	19·7	11·6	18·1	25·9	15·7	4·6	0·2	100

* Age–sex standardized rates.

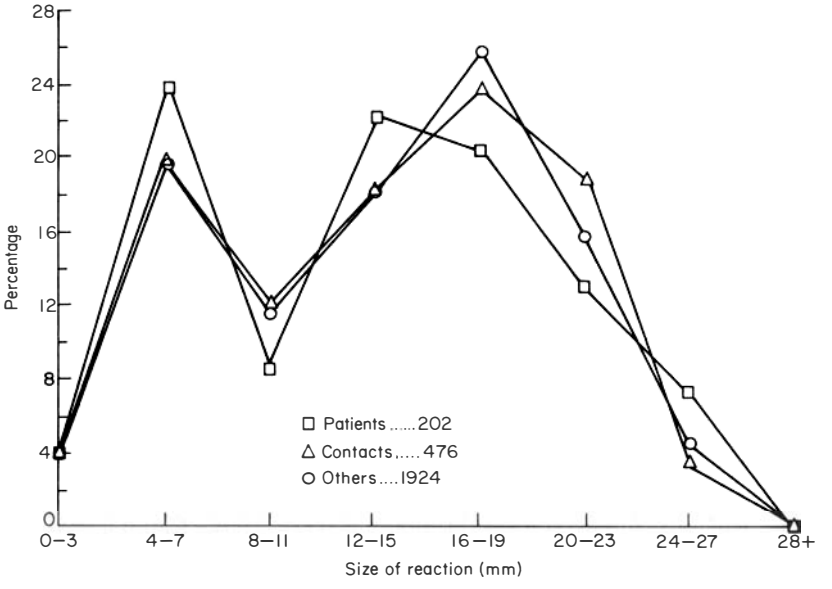

Figure 3. Distributions (age–sex) of patients, contacts and others by size of reaction to Rees antigen.

In the findings reported here, care was taken to avoid the methodological problems on account of batch-to-batch variations and variations due to multiple testers and readers. We found a positive and very high degree of correlation between reactions to the Rees and Convit antigens. The findings were similar in different age and sex groups as well as in patients and contacts of the patients. Since the two antigens were calibrated to different standards, the mean difference of 1·2 mm in the skin-test reactions to the Rees and Convit antigens may just be a dose effect and as such not biologically significant. For the various results presented, for patients, contacts and population, the information is given on the Rees antigen, but the results were very similar for the Convit antigen as well.

In the study reported here, we used CD-19 batch for the Rees antigen and SA-IND, 1-16-86 for the Convit antigen. We have observed earlier, in two independent experiments, that the correlation between reactions to two different batches (CD-19, CD-73) of the Rees antigen was much stronger ($r = 0{\cdot}81$ and $0{\cdot}77$) than that observed for two different batches (SA-IND, 1-16-86 and IB-Lote, 4-6-87) of the Convit antigen ($r = 0{\cdot}53$ and

0·66).[10] One study[12] observed a positive correlation ($r = 0·7$) between postvaccination reactions to Rees (CD-19) and WEL-1 (an antigen prepared using the protocol for Convit antigen) antigens although the responses to WEL-1 antigen were uniformly lower than that to the Rees antigen. They also found that the WEL-1 antigen did not produce pre-vaccination induration in the vast majority of the individuals tested. The protein content of WEL-1 and Rees antigens was 0·5 mcg and 1·0 mcg per dose, respectively.[12] It is difficult to say whether the high degree of correlation observed by us between reactions to Rees and Convit antigens was limited to the particular batches used or the observed dissimilarity on the other occasions was due to variations in the calibration procedures for the Convit antigen. Considering the magnitude of positive correlation observed between reactions to the two antigens, both by us and by Ponnighaus and Fine, the observed differences might be on account of dose or calibration effect.

In the 888 individuals skin tested with Rees, Convit and tuberculin antigens, a low level of positive correlation was seen between reactions to the Rees antigen and tuberculin, as well as between reactions to the Convit antigen and tuberculin. It was seen that this association was significant in the younger age group of 1–9 years only. However, even in this age group as many as 24% of children who were tuberculin negative showed positive reactions to the Rees antigen. It appears that the tuberculin status of an individual would affect the Rees antigen positivity only marginally and also only in the younger children in the study area. Thus tuberculin positivity was at most only partially responsible in producing cross-sensitization to the Rees antigen. It was also seen that prior tuberculin testing did not seem to influence the pattern of reactions to a subsequent test with the Rees antigen.

The differences observed in the two villages with respect to the Rees and the Convit antigen reactions were marginal, though statistically significant (Table 3). The frequency curves, however, followed the same pattern and in all probability the observed difference did not have any biological significance.

The BCG scar status did not influence reaction to Rees antigen in our study. Convit and Zuniga in Venezuela and Fine and Ponnighaus in Malaŵi found cross-sensitization on account of BCG scar status.[7] One study[13] in Sri Lanka did not find any such influence, another study[14] in Agra, India observed that the previous BCG scar status did not influence the leprosin-A (Rees antigen) results in the children studied. However, in a prospective study, they found an increase in the Rees antigen positivity following BCG vaccination.[14] Also, it was seen in our study that even the tuberculin reactions did not depend on the BCG scar status. However, it should be noted that the population in Chengalpattu District was kept free from BCG vaccination programme, and the proportion of persons with a BCG scar was less than 10%. The practice generally followed in India is BCG vaccination at birth. The waning of tuberculin sensitivity after BCG has been documented in Chengalpattu District.[15–16] In a study in Madras city, it was observed that the post-BCG tuberculin sensitivity in newborn babies waned considerably over a period of 12 months.[17] In a study in Sri Lanka involving 740 healthy children given a BCG vaccination in their first month, it was seen that 80% of the children showed tuberculin anergy in spite of having a visible scar.[18] A similar waning effect may also be expected on cross-sensitization, if any, for the Rees antigen.

We obtained a consistent bimodal pattern with both the Rees and the Convit antigens (Figure 1). Based on this pattern, we considered reactions of 12 mm or more as 'positive'. With this cut-off point, 73% (138 of 188) of patients with paucibacillary leprosy were Rees

antigen positive while 71% (10 of 14) of patients with multibacillary leprosy were Rees antigen negative. The cut-off point used by various workers for defining positivity have been different. In the Malawian studies, despite observing a clear bimodal distribution, the investigators arbitrarily called reactions of > 5 mm as 'positives'.[12] The criterion for positivity adopted for the Convit antigen reactions by Convit and his colleagues was 10 mm or more.[19] Stanford and Lema considered indurations of 2 mm or more as a positive response.[20] Different definitions adopted by various investigators will certainly contribute to the observed differences with respect to positivity. The frequency distributions of reactions to the soluble antigens appear to follow different patterns in different areas.[13,21,22] Prevalence of environmental mycobacteria and other organisms, prior vaccination with BCG as well as infection with *M. leprae* are some of the likely causes to affect these distribution patterns.

Results from earlier studies indicate that these antigens might be useful for classification of leprosy and in identifying the population at a high risk for multibacillary leprosy. Data from Malaysia and India showed that patients with lepromatous leprosy were uniformly negative to Rees antigen while tuberculoid patients were positive.[7] Samuel *et al.* have concluded from their work in five different countries, namely India, Uganda, Kenya, Nepal and Bhutan, that the Rees antigen (Leprosin-A) reactions were positive in the high resistant forms of leprosy and were negative in low resistant lepromatous forms.[23] Ponnighaus and Fine also found that 80% of untreated paucibacillary patients with leprosy in Malawi gave positive skin test reactions (> 5 mm) to a Rees-type antigen, whereas, only 35% of a sample of treated patients were positive. All the multibacillary patients, although only a few, were skin test negative.[7] Convit had reported similar results with his antigen in Venezuela.[7] These findings with the soluble antigens are similar to the Mitsuda reaction to lepromin test in leprosy patients.

In the present study we noticed that Rees antigen positivity was 64·0%, 70·7% and 76·2% in neuritic, indeterminate and the TT–BT groups of patients respectively. In the BL–LL groups of patients, however, it was only 28·6%. Thus the skin test results in the study patients from South India followed a similar pattern as reported by other workers. However, the capacity of the Rees antigen to classify the leprosy patients into two groups, paucibacillary and multibacillary, was limited and the dividing line was blurred. Fifty (27%) of the 188 paucibacillary patients were 'negative' and 4 (29%) of the 14 multibacillary patients were 'positive' to the Rees antigen test.

We observed that the distributions of reactions to both the Rees and Convit antigens were bimodal. This observation was true for patients with leprosy, their household contacts, as well as the general population. It was seen that the reaction positivity to the Rees antigen increased sharply with the increase in age in both males and females. Generally, the reaction positivity was higher in males than in females. It is to be expected that the risk of infection will increase with age. However, it is difficult to conceive such a steep rise with age on account of the new infections due to *M. leprae* alone. The similarity in the distributions of reactions to Rees antigen in patients with leprosy, their household contacts and general population became strikingly demonstrable when the age and sex standardized proportions in different reaction sizes were considered (Table 5 and Figure 3). The Rees reaction positivity was also very similar in patients, contacts of both paucibacillary and multibacillary patients with leprosy, and general population in different age groups (Figure 2). In this respect, our results are different from the observations of Convit and Zuniga in Venezuela. Convit and Zuniga extensively used the

M. leprae soluble antigen (Convit antigen) in Venezuela. Their initial objective was to have a test comparable to the lepromin test. They also hoped that the Convit antigen would be useful to determine prevalence and incidence of infection due to *M. leprae*. They found that the prevalence of Convit skin test positivity correlated with the level of prevalence of leprosy in the population. Only 3·5% individuals were positive in nonendemic areas of Chile compared to 46% positives in endemic areas of Venezuela. Contacts of leprosy patients showed higher levels of positivity than that in the general population.[7] Extensive studies have also been done in Malaŵi using the *M. leprae* soluble antigens. Fine *et al.* found a clear bimodal distribution of the skin reactions in the Northern Malaŵi population endemic for leprosy. Based on the rising prevalence rate of positivity with age, they postulated that the skin tests were specific for some mycobacterial experience.[21]

In a study in Bangladesh, Cree *et al.* observed that the Rees antigen (CD-19) positivity ($\geq$5 mm) was similar in 78 household contacts of untreated patients, 34 untreated paucibacillary patients and 50 randomly selected indigenous subjects. It was 56·6%, 56·3% and 55·6% in the three groups respectively.[24]

In a population based study in Sri Lanka, Pinto *et al.* did not find any statistically significant changes in Rees' antigen (CD-19) positivity with respect to age, sex, race or BCG vaccination status.[13]

It is difficult to explain the differences observed in the results of these studies. However, the possible reasons could be the use of different batches of antigens, prevalence of different levels of leprosy endemicity and nonspecific sensitization, as well as the differences in the populations studied.

Variations observed in some of the studies mentioned above could be explained as due to the substantial amount of variations in the skin reactions produced by different batches of these antigens. Such batch-to-batch variations have been observed and documented by us.[10] Similar observation is also reported by Fine *et al.* in the Malaŵi studies.[21] Considerable batch-to-batch variability of skin test antigens was also observed in Venezuela.[7] Rees antigen batch CD-19 was used by us in the study reported here. This batch has been extensively used in different parts of the world. Pinto *et al.*,[13] Cree *et al.*[24] and Ponnighaus & Fine[12] used the same batch in their studies in Sri Lanka, Bangladesh and Malaŵi respectively. The results in these studies varied with respect to the frequency distributions of skin test responses in different groups of populations. The batch-to-batch variations alone, therefore, cannot explain the different patterns of skin test responses to these antigens.

It was also noted that the skin test indurations produced by the Rees and Convit skin tests were extremely soft and needed a considerable amount of training even for standard readers experienced in reading tuberculin reactions.[10] Ponnighaus & Fine, using Rees antigen (batch CD-19) found almost spontaneous conversions and reversions in a substantial proportion of the subjects retested.[12] This problem would also therefore contribute to the differences observed in the various studies.

The leprosy epidemiological situation in Venezuela is different in comparison to that in other areas. Leprosy prevalence in Venezuela is relatively low. There was only one active case under control per thousand population in 1981.[25] In Venezuela it is reported that the leprosy problem has rapidly declined over the past three decades and that there is a relative increase in the proportion of multibacillary cases.[25] In the Malaŵi vaccine trial area, prevalence of clinical leprosy is found to be about 5 per thousand and multibacillary

cases constitute about 7% of these cases.[26] In our study area the prevalence of leprosy is around 40–50 per thousand population and about 7% of these cases are of the multibacillary type.

Categorization of the population into leprosy patients, their contacts and general population in the present study was based on a prevalence survey. It is possible that some individuals who might have been leprosy cases and in whom leprosy lesions had completely resolved at the time of the prevalence survey were considered as non-cases. The majority of the leprosy patients in the study were of the paucibacillary type, only 7% belonged to the multibacillary type. As such the majority of the 'contacts' belonged to households with paucibacillary patients. However, the pattern of reactions in contacts of multibacillary patients was similar to that in contacts of paucibacillary patients. Since the number of contacts of patients with multibacillary leprosy was small and Rees antigen positivity in them was not significantly different from that in other contacts, all the contacts were considered as one group.

Our study area is adjacent to Thiruvallur Taluk in Chengalpattu District, where a BCG prophylaxis trial against tuberculosis and leprosy was conducted, and which had a high prevalence of nonspecific sensitivity.[15] Thus the study area is expected to have a high level of nonspecific sensitization. Studies carried out in different parts of India have demonstrated that nonspecific sensitization is not restricted to Chengalpattu District alone, but is a widespread phenomenon. In the temperate zone the population at higher altitudes had a lower prevalence of nonspecific sensitivity than that among the population in the plains. In the tropical zone, prevalence of nonspecific sensitization was high,[27–29] however, even in Kashmir valley, situated about 1650 m above the mean sea level, prevalence of nonspecific sensitivity was 59%.[30] It is difficult to say to what extent the existing high level of nonspecific sensitization in the study area would have influenced the pattern of results to the skin-test antigens observed by us.

To sum up, the results of the study reported here clearly show that the Rees and the Convit antigen skin tests are not sensitive enough to detect leprosy, they do not appear to be specific enough to confirm the clinical diagnosis of leprosy. Since the present study was conducted in a leprosy endemic area, the differences between contacts of leprosy patients and the general population might not be evident. Almost every individual in the study population was exposed to the *M. leprae* infection. However, the pattern of reactions to these antigens seen in patients and the almost identical pattern observed in other population groups, raise serious doubts about their use in detecting *M. leprae* infection in a leprosy endemic population with a high prevalence of nonspecific sensitivity.

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