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The Relationship between Reactivities to Lepromin A (Fernandez and Mitsuda) and a Soluble Protein Antigen of *Mycobacterium leprae*

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SUMMARY Three methods of evaluation were used to investigate the relationship between skin test reactions elicited by different antigens of *Mycobacterium leprae*. The latter were the Fernandez and Mitsuda reactions to lepromin, and that to a Soluble Protein Antigen (SPA) of *M. leprae*. All three methods of evaluation demonstrated some degree of relationship though not as high as would be expected. The closest correlation was between Mitsuda and SPA reactions; while Fernandez and Mitsuda, and Fernandez and SPA reactions showed more or less similar coefficients of correlation.

Lepromin produces two types of reactivity – one of the Fernandez type and the other, of the Mitsuda type. These two reactivities have been considered to be of different aetiological and immunological significance. As such, the relationship between these two would be of interest. Hence, from the earliest days of lepromin use, the relationship between the reactivities of lepromin and also tuberculin have been variously investigated. However, there are yet considerable gaps in the literature. The earlier surveys all used lepromins of human origin, and there is no literature with lepromins of armadillo origin, which have been introduced recently. Further, a Soluble Protein Antigen (SPA) of *Mycobacterium leprae*, has also been introduced recently, for use in skin testing in relation to leprosy. The literature on this test is as yet limited and its correlation with the other reactivities is not well defined. In this report is presented an examination of the relationship between different types of reactivity to Lepromin and SPA, from a skin test survey in Sri Lanka.[1,2]

MATERIALS AND METHODS

The tests done have been described in detail elsewhere.[1,2] The methodology was as follows.

The method of skin testing used was the standard intradermal technique recommended for tuberculin testing.[3] The skin tests were carried out on the volar aspect of the forearm, at sites 6-7 cm apart. All individuals tested were adults (12 years of age and above), in four population groups, in three geographical areas. The antigens used in skin testing were (1) Lepromin A (of armadillo origin – kindly supplied by Dr. Hastings of the National Hansen's Disease Center, Carville, USA, through courtesy of the Chief, Leprosy Section, World Health Organisation, Geneva.), (2) a Soluble Protein Antigen (SPA) of *M. leprae*, prepared by ultrasonic disruption of the organism, with a protein content of 10μg/ml (batch CD19) (kindly supplied by Dr. R.J.W. Rees of the IMMLEP *M. leprae* Bank, Harrow, Middlesex, UK.) and (3) Tuberculin PPD – RT 23, 2 T.U. per dose (Staten Serum Institut, Copenhagen, Denmark). With lepromin A, Fernandez reactivity was read at 48 hours and Mitsuda reactivity at 28 days. With SPA and tuberculin reactivities, the reactions were read at 72 hours. The results were read as the maximum transverse diameter of induration palpated, with Fernandez, SPA and tuberculin reactivities, and that of the nodule palpated (observed) of Mitsuda reactivity.

Results were available for reactivities elicited by three different antigens in four population groups, in three geographical areas (Table 1). The correlations examined were between Fernandez, Mitsuda and

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Table 1 Availability of results of different types of reactivity

Location of population group	Antigens used in tests	Comparison of reactivities possible	Numbers tested				
			Total	BCG Status			
				Negative		Positive	
				Male	Female	Male	Female
Nuwara Eliya - Pedro (elevation 1950m)	(1) Lepromin A (bacillary content 3×10^7/ml) (2) Tuberculin PPD RT23 (1 t.u./dose)	(1) Fernandez *vs.* Mitsuda (2) Fernandez *vs.* Tuberculin (3) Mitsuda *vs.* Tuberculin	116	22	44	20	30
Pussellawa (elevation 900m)	(1) Lepromin A (as above) (2) Tuberculin (as above)	(1) Fernandez *vs.* Mitsuda (2) Fernandez *vs.* Tuberculin (3) Mitsuda *vs.* Tuberculin	195	26	85	30	55
Nuwara Eliya - Mahagastota (elevation 1950m)	(1) Lepromin A (bacillary content 4×10^7/ml) (2) Soluble Protein Antigen of *M. leprae* (SPA) (3) Tuberculin (as above)	(1) Fernandez *vs.* Mitsuda (2) Fernandez *vs.* SPA (3) Mitsuda *vs.* SPA (4) SPA *vs.* Tuberculin (5) Fernandez *vs.* Post-lepromin Scar (read at 7 months) (6) Mitsuda *vs.* Post-lepromin Scar (read at 7 months) (7) SPA *vs.* Post-lepromin Scar (read at 7 months)	137 184 128	18 29 21	59 77 53	23 26 20	37 52 34
Galagedera (elevation 150m)	(1) Lepromin A (bacillary content 4×10^7/ml) (2) SPA	(1) Fernandez *vs.* Mitsuda (2) Fernandez *vs.* SPA (3) Mitsuda *vs.* SPA (4) Fernandez *vs.* Post-lepromin Scar (read at 3 months) (5) Mitsuda *vs.* Post-lepromin Scar (read at 3 months) (6) SPA *vs.* Post-lepromin Scar (read at 3 months)	218 217 178	35 44 25	83 79 75	34 33 23	66 61 55

SPA reactivities. The relationship of the post lepromin scar to different types of reactivities had been presented elsewhere and was not considered here.

RESULTS

The simplest way in which the relationship between two types of reactivities may be examined is by the use of bivariate frequency distributions; such distributions are presented in Tables 2, 3 and 4.

The correlations between the different types of reactivities (in the whole populations tested) were evaluated using the technique of regression analysis. The results of this evaluation (Table 5) showed that all three types of reactivities are significantly correlated ($\alpha > 0.01$). The highest degree of correlation (r^2) was found between Mitsuda and SPA, followed by Fernandez and SPA, and Fernandez and Mitsuda (both more or less equal). However, r^2 is low suggesting that the degree of correlation between the reactivities is low.

From earlier work it was shown that BCG vaccination status and location may be variables which effect reactivities of different types with *M. leprae* (while race, sex and age – of those above 12 years, did not).[1,2] Further, it was also shown that non-reactors and reactors,[1,2] may show differences in immunological responsiveness.[5,6] (See note 'a'). The influences of all these variables on the correlation between different reactivities were also studied by examination of different subsets (by use of regression analysis). From the mass of data obtained (summarised in Table 6), some trends may be observed.

Note 'a' - A "non-reactor" was defined as an individual who showed a reaction of 2 mm or less with Fernandez and Mitsuda reactivities, and 4 mm or less with SPA reactivity. A "reactor" was an individual who showed a reaction of 3 mm or more with Fernandez and Mitsuda reactivities and 5 mm or more with SPA reactivity.

Table 2 Bivariate frequency distribution of Fernandez and Mitsuda reactivities of the whole population

Reaction sizes with		Mitsuda reactivity										
		0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
Fernandez reactivity	0	47	1	21	43	32	5	2				
	1-2	34	5	17	22	16	5		1			
	3-4	18	5	17	28	23	5	2				
	5-6	9	4	16	41	30	10	4	1			
	7-8	9	2	7	19	18	8	3	2	1		
	9-10	4	1	6	20	16	11	4	1	1		
	11-12	2		5	9	6	13	2	2			
	13-14	2		4	4	3	4	1	1			
	15-16				1	1	2	1				
	17-18	2		2		1	1					
	19-20											

Note: One person had a reaction size of 25 mm with the Fernandez reaction and 19 mm with the Mitsuda reaction.

Table 3 Bivariate frequency distribution of SPA and Fernandez reactivities of the whole population

Reaction sizes with		Fernandez reactivity										
		0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
SPA reactivity	0	47	36	26	12	7	3	1	1			
	1-2	3	5	4	1	1						
	3-4	1	4	1	1	1		1				
	5-6	1	2	6	2	3	1					
	7-8	4	5	9	8	4	3	2	1			
	9-10		11	11	15	9	9	1			1	
	11-12	7	8	8	13	8	16	8	2	2		1
	13-14		1		8	8	7	6	1	1	2	
	15-16		1	1		1	3	3	3	1		
	17-18		1		1	2	2	5				
	19-20						1	2				

Note: Three persons had reaction sizes of 13, 21 and 22 mm with the SPA reaction, and 25, 9 and 11 mm, respectively, with the Fernandez reaction.

Table 4 Bivariate frequency distribution of SPA and mitsuda reactivities of the whole population

Reaction sizes with		Mitsuda reactivity										
		0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
SPA reactivity	0	50	5	15	27	7	3					
	1-2	10		1	2							
	3-4	1		1	3	1						
	5-6	2	1	2	6	1						
	7-8	6	1	4	8	4	3	2				
	9-10	6	1	7	13	11	2					
	11-12	5		6	14	11	14	1	1			
	13-14	2		3	3	8	3	4				
	15-16	1		1	1	2	5	1	2			
	17-18				2		2	1	2	1		
	19-20					2						

Note: Two persons had reaction sizes of 21 mm each with the SPA reaction and 0 and 5 mm, respectively, with the Mitsuda reaction.

Table 5 Correlation between different types of reactivity of the whole population

Subsets correlated	Constant (S.E.)	Coefficient (S.E.)	r^2	F Value
Fernandez *vs.* Mitsuda	2.4870 (0.280)	0.4300 (0.045)	0.34	91.02**
Fernandez *vs.* SPA	1.9155 (0.275)	0.4573 (0.031)	0.36	218.20**
Mitsuda *vs.* SPA	2.7404 (0.2482)	0.3253. (0.027)	0.51	135.50**

**Statistically significant at $\alpha \geqslant 0.01$

Table 6 Correlation between different reactivities to antigens of *M. leprae,* considered in relation to non-reactor/reactor status of each reactivity (levels of significance)

Subset				F *vs.* M where F is NR/R	F *vs.* M where M is NR/R	M *vs.* S where M is NR/R	M *vs.* S where S is NR/R	F *vs.* S where F is NR/R	F *vs.* S where S is NR/R	F *vs.* M where S is NR/R	M *vs.* S where F is NR/R	F *vs.* S where M is NR/R
Of whole population (Total)		NR		NS	NS	NS	NS	**	NS	**	*	**
		R		**	**	**	**	**	**	**	**	**
Effect of BCG Status		NR	BCG negative	NS	NS	NS	NS	**	NS	NS	**	*
			BCG positive	NS	NS	NS	NS	NS	NS	NS	**	*
		R	BCG negative	**	**	**	**	**	**	**	**	**
			BCG positive	**	**	**	*	**	**	**	**	**
Effect of location	BCG negative	NR	Pussellawa	NS	NS							
			Pedro	NS	NS							
			Mahagastota	NS	NS	NS	NS	NS	*	NS	*	*
			Galagedera	NS	NS	NS	NS	**	NS	NS	NS	*
		R	Pussellawa	NS	NS							
			Pedro	NS	NS							
			Mahagastota	NS	**	**	*	**	**	NS	**	**
			Galagedera	**	**	**	**	**	**	**	**	**
	BCG positive	NR	Pussellawa	NS	X							
			Pedro	NS	NS							
			Mahagastota	NS	NS	NS	NS	NS	NS	NS	**	NS
			Galagedera	NS	X	X	NS	NS	NS	NS	**	NS
		R	Pussellawa	NS	**							
			Pedro	NS	NS							
			Mahagastota	**	**	*	*	**	*	*	**	**
			Galagedera	**	**	**	NS	**	*	NS	*	**

NR – Non-reactor; R – Reactor; NS – Not significant; * – Significant at $\alpha \geqslant 0.05$; ** – Significant at $\alpha \geqslant 0.01$;
X – Numbers insufficient for assessment; F – Fernandez reactivity; M – Mitsuda reactivity; S – SPA reactivity

(1) With respect to non-reactor/ reactor status of the different types of reactivities, (See note 'b'), where the status considered is that of an antigen concerned in the correlation being evaluated, the non-reactors seem to show no significant correlation with the reactions of the other reactivity while the reactors frequently showed significant relationships. However, this trend is not as clear when the determining variable, is the immunological status of an antigen other than those between which the correlation is being evaluated. In this evaluation it should be noted that the non-reactors of the determining variable were limited in extent (for Fernandez and Mitsuda reactivity 0-2 mm reactions and for SPA reactivity 0-4 mm reactions), while the reactors of the determining variable were of greater extent-reactions of 3 mm and 5 mm or more respectively.

Thus it is possible that the limited availability of results with the non-reactor components (of the determining reactivity) affected the observed correlations. And, that this possibility carries some weight, is indicated by the observation that more subsets, show results with significant correlations where the determining non-reactor status is of a reactivity, other than the two whose correlation is being evaluated. Yet, even in the latter situation, the frequency with which significant (correlations) results are obtained in the non-reactor groups is not as high as those with the reactor groups.

(2) BCG vaccination status *per se,* and location, do not seem to produce any marked change in the level of significance (or not) of the correlations observed.

At two locations, Mahagastota and Galagedera, the results of three reactivities, (Fernandez, Mitsuda and SPA), were available in the same individuals. Thus, the relationships of each of these reactivities with each other could be evaluated in a multiple regression analysis. Since there is no data on which to base a decision as to which of these three reactivities be considered the dependent and independent variables, an arbitrary decision was made to consider Fernandez reactivity as the dependent variable. Correlations were computed for different statuses (*i.e.* non-reactor/ reactor) of each of the three types of reactivities, based on the following model

$$F = a + b(M) + c(S) + E$$

where F = Fernandez reaction size, a = constant, b and c = regression coefficients, M = Mitsuda reaction size, S = SPA reaction size and E = the error.

The results of this analysis revealed that Fernandez and SPA reactivities showed different types of relationships as compared with Mitsuda reactivity, when non-reactor/ reactor status was considered. Both Fernandez and SPA showed no significant relationship in the non-reactor groups while Mitsuda reactivity did. All three reactivities showed significant relationships in the reactor groups.

Another view of the relationships between reactivities may be obtained by examination of the frequency distributions of reactions of one type of reactivity in relation to those of another type, the latter categorised into non-reactor/reactor groups – the determining variable; (see explanatory notes 'a' and 'b'), for example, the frequency distributions of Mitsuda reactions, in those whose Fernandez reactions were 2 mm or less (non-reactors) and 3 mm or more (reactors). The majority of frequency distributions (Figs. 1-4), thus constructed, showed (ten of the sixteen, definitely) a difference in pattern of distribution between those in the non-reactor and reactor groups (of the determining reactivity). Thus, the distribution of the latter reactors showed a "shift to the right", as compared with those of non-reactors (with increase of mean reaction size, and reduction of the non-reactor component of the frequency distribution).

A third and simple way of looking at the relationship between reactivities (used by most workers who have investigated correlations) is to look at the occurrence of "negatives" and "positives" with the different reactivities in relation to each other. However, one problem here, is that different workers have used different qualitative and numerical criteria for the definition of "positive". The criteria used here were as described elsewhere,[1,2] *i.e.,* 3 mm or more for "positive" or "reactor" in Fernandez and Mitsuda reactivity and 5 mm or more for SPA reactivity.

It was found that with Fernandez and Mitsuda reactivity, 65% yielded results which tallied, in so far as non-reactor/reactor (negative/positive) status was concerned. Examination of the results also revealed the influence of BCG vaccination on reactivity status to be seemingly more marked for Mitsuda than for Fernandez reactivity.

When the reactivities to Fernandez, Mitsuda and SPA were examined together at the two locations where the results of all three were available, agreement of status was found in 60%. Here again, the influence of BCG vaccination on results could be observed. Where the increase

Note 'b' - In the examination of the relationship between two reactivities, the distribution of reactions of one reactivity, in those individuals in whom another reactivity showed a non-reactor status (or alternatively a reactor status), could be examined. The latter reactivity could be described as the determining reactivity or variable. In the examination of a bivariate frequency distribution, such a determining variable can be one of the reactivities being examined. Also a bivariate frequency distribution can be constructed for two reactivities, where a third reactivity was the determining variable, and analyses carried out on the frequency distributions, so constructed.

of reactors was most marked with vaccination was with Mitsuda reactivity (approx. 33%), followed by SPA (23%) and then Fernandez (4.5%). Statistical evaluation of the occurrence of non-reactor/reactor status in relation to individual Fernandez and Mitsuda reactions, with BCG vaccination showed that in all cases reactors were found in higher numbers following BCG vaccination ($\alpha \geqslant 0.01$).

In two locations (Mahagastota and Galagedera) the effect of BCG vaccination on the occurrence of different Fernandez, Mitsuda and SPA reactivity statuses (ranging from all reactors to all non-reactors) could be evaluated. Again, both locations showed distinctive patterns with the different reactivity status combinations. However, the proportionate occurrence of each combination with different BCG vaccination statuses in the two locations were different, suggesting possible geographic differences. Further, again it was seen that the effect of BCG vaccination on SPA reactivity status resembled the effect of BCG vaccination on Mitsuda in that the occurrence of reactor status was increased markedly. This differed from the situation with Fernandez reactivity.

DISCUSSION

From the foregoing analysis it can be seen that all three types of reactivity to antigens of *M. leprae* are significantly correlated with each other ($\alpha \geqslant 0.01$). This high level of correlation was demonstrated by all three of the methods of evaluation used. The degree of relatedness between reactivities is unexpectedly low and varied. The closest correlation among reactivities of antigens to *M. leprae* was between Mitsuda and SPA.

It has been shown[5,6] that non-reactor and reactor groups with different reactivities tend to behave differently in various situations suggesting that they probably are of (im-

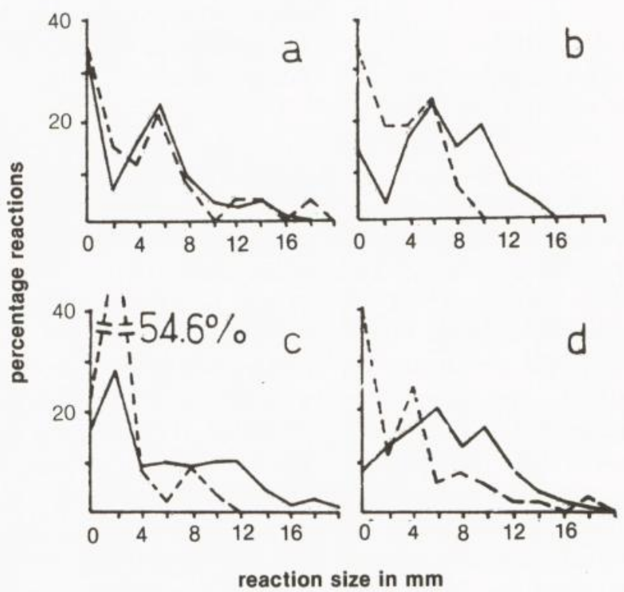

Fig. 1 Distribution of Fernandez reactions where Mitsuda reaction size is ⩽ 2 mm (--------) and ⩾ 3 mm (———) at Pussellawa (a), Pedro (b), Mahagastota (c) and Galagedera (d).

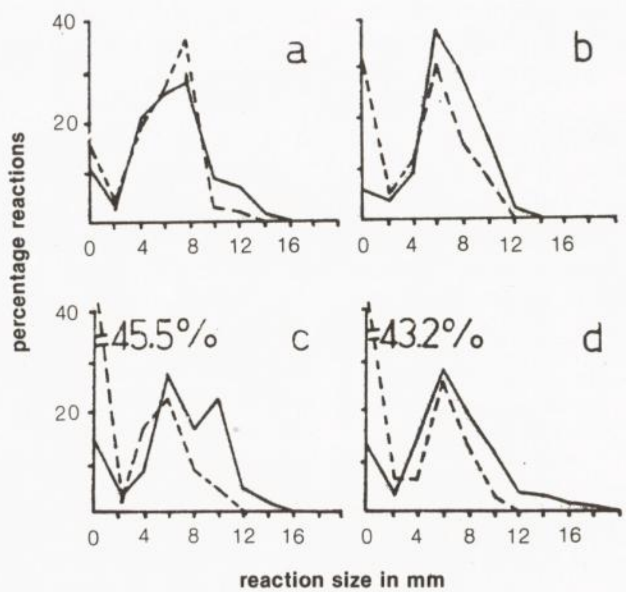

Fig. 2 Distribution of Mitsuda reactions where Fernandez reaction size is ⩽ 2 mm (--------) and ⩾ 3 mm (———) at Pussellawa (a), Pedro (b), Mahagastota (c) and Galagedera (d).

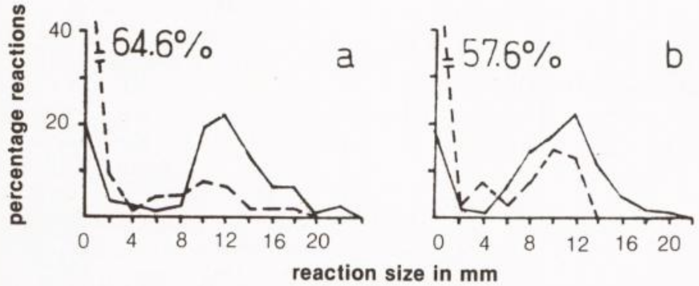

Fig. 3 Distribution of SPA reactions where Fernandez reaction size is ⩽ 2 mm (--------) and ⩾ 3 mm (———) at Mahagastota (a) and Galagedera (b).

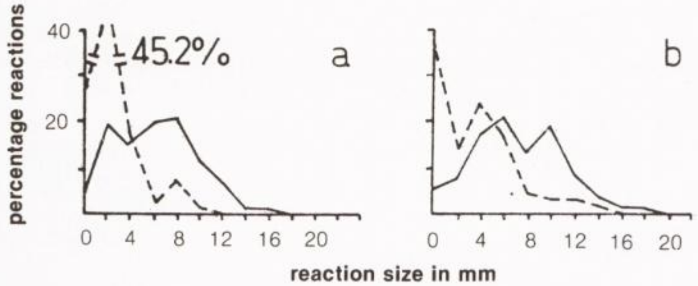

Fig. 4 Distribution of Fernandez reaction where SPA reaction is ⩽ 4 mm (--------) and ⩾ 5 mm (———) at Mahagastota (a) and Galagedera (b).

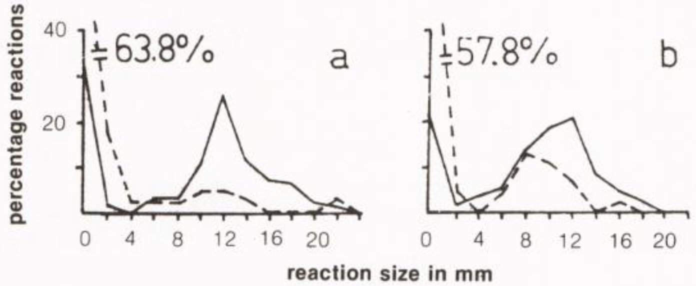

Fig. 5 Distribution of SPA reactions where Mitsuda reaction is ⩽ 2 mm (--------) and ⩾ 3 mm (———) at Mahagastota (a) and Galagedera (b).

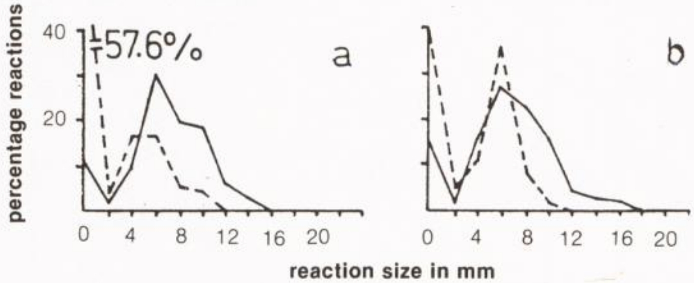

Fig. 6 Distribution of a Mitsuda reactions where SPA reaction is ⩽ 4 mm (--------) and ⩾ 5 mm (———) at Mahagastota (a) and Galagedera (b).

munologically) different cohorts. This study adds further evidence to support that contention from examination of the correlations between non-reactor and reactor groups of one reactivity with the reactions of another reactivity.

A third area where evaluation of correlations yielded data, was on the influence of BCG vaccination on different types of reactivity. Here, it was seen that BCG vaccination caused a similar proportional increase in reactors with both Mitsuda and SPA reactivity, but to a markedly lesser and almost insignificant extent, with Fernandez reactivity. In this respect too, a similarity was seen between Mitsuda and SPA reactivity.

The findings of this study are thus somewhat unexpected. On theoretical grounds; it would be expected that SPA and Fernandez reactivity would show identical reactions or at least a very close correlation between reactions; but this was not so. These findings also pose questions as to the significance of each type of reactivity.

It is generally held that Fernandez reactivity in leprosy is the analogue of tuberculin reactivity in tuberculosis. The use of SPA is based on the possibility that it may evoke a tuberculin like response to a greater degree than may the Fernandez reaction with lepromin. In a recent report, Convit *et al.*[7] showed that the cell free supernatant of lepromin was the fraction that elicited the Fernandez reaction. The cell free supernatant of lepromin would be expected to have similar antigens to SPA of *M. leprae,* but examination of the correlations does not support this hypothesis.

On the other hand, Mitsuda reactivity is considered to be a "vaccination type" response similar to that seen following BCG vaccination. Thus, it may both measure and demonstrate preexisting hypersensitivity. As far as may be determined at present, SPA measures and demonstrates the former but does not induce hypersensitivity. Thus, the correlation between SPA and Mitsuda reactivity may be considered as evidence that Mitsuda reactivity demonstrates and measures preexisting hypersensitivity as well.

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