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The detection of subclinical leprosy using a monoclonal antibody based radioimmunoassay

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Summary The monoclonal antibody based competition radioimmunoassay test was used to examine sera from 100 healthy household contacts of known leprosy patients. Only 6 out of 100 contacts had detectable specific antibodies.

It remains conjectural that this small fraction of contact subjects may be at much higher risk of developing disease than those without antibodies. Contacts who are antibody positive and lepromin negative (as were 4 of the 6), would best qualify for being offered chemoprophylaxis.

Introduction

Populations in endemic areas are exposed to the risk of developing leprosy because of delayed presentation of cases and prolonged infectivity before treatment is commenced. A large proportion, probably over 90%[1] of those exposed will develop subclinical infection but only a much smaller proportion will subsequently develop clinical leprosy;[2] the majority of subclinical infections are eradicated by an effective cell-mediated immune response to *Mycobacterium leprae*. A screening test, specific and predictive for leprosy is needed in order to reduce the large at-risk population to a small high-risk population which would be feasible to follow up with chemoprophylactic treatment.

Tests based on the cell-mediated response to *M. leprae* are not sufficiently specific because of cross-reactivity with *M. tuberculosis* and environmental mycobacteria.[3] However, a new serological test for leprosy has been developed following the production of a monoclonal antibody, ML04, to the 35 K protein

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antigen of *M. leprae*. This was found to be fully specific for leprosy when tested against patients with active pulmonary tuberculosis, autoimmune diseases, carcinoma and healthy controls.[5] Because of this high specificity this test was used to screen a population of healthy contacts of known leprosy source cases.

Materials and methods

One hundred household contacts of leprosy patients were studied. The contacts consisted of 84 relatives of patients attending the Central JALMA Institute for Leprosy, Agra and 16 relatives of patients attending the rural health centre at Deeg, Rajasthan. The contacts were all examined and excluded from the trial if they had any definite or probable clinical signs of leprosy. Each contact had a lepromin test performed and blood was taken for antibody titre determination. The diagnosis of leprosy and classification according to the Ridley–Jopling scale[6] was made for each patient on the basis of clinical features, slit-skin smear and lepromin test in all cases and in addition histology of the skin lesion in 12 cases.

LEPROMIN TEST

Dharmendra lepromin[7] was used to assess the skin delayed hypersensitivity to *M. leprae* in each contact. A positive early reaction (Fernandez reaction) was recorded after 48 hours if there was erythema and induration of 5 mm or more in diameter; a positive late reaction (Mitsuda reaction) was recorded at 4 weeks if there was a papule of 4 mm or more in diameter.

SERUM ANTIBODY COMPETITION TEST (SACT)

Antibodies to the 35K antigen (MY2a epitope) of *M. leprae* were detected by radioimmunoassay. The principle of the test is competition between ^{125}I labelled *M. leprae* specific ML04 antibodies and homologous human antibodies present in the test serum for antigen binding. The technique followed was as described by Sinha *et al.*[5] Results were expressed as the reciprocal of the serum dilution needed to inhibit 50% (ID_{50} value) of ^{125}I-ML04 binding to the antigen.

Results

Six out of 100 household contacts of leprosy patients had antibodies to the MY2a epitope of *M. leprae* as determined by the SACT assay. All 6 SACT positive cases had low titres of antibody with an ID_{50} value of 5. Figure 1 shows the pattern of inhibition of ^{125}I-ML04 binding by the dilutions of the antibody positive sera. The hatched area indicates the range of inhibition by various control sera including those of tuberculosis patients.

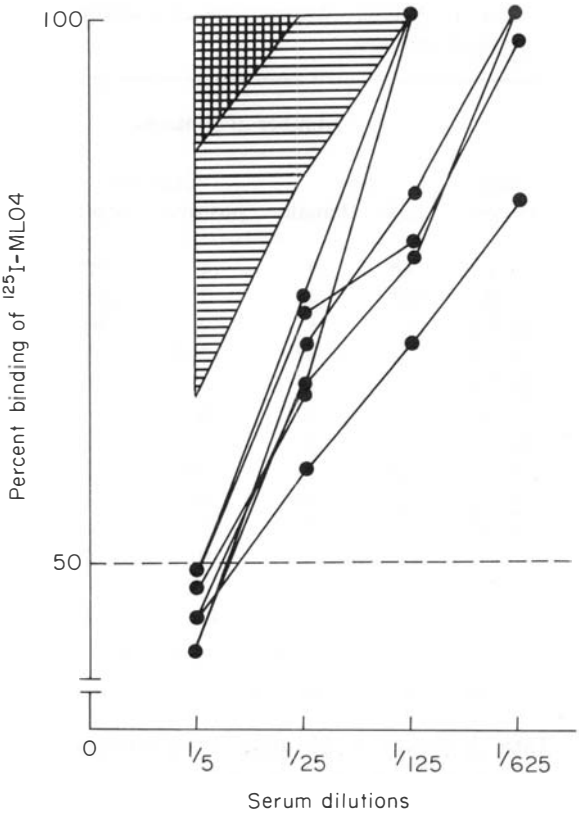

Figure 1. ^{125}I-ML04 binding of diluted sera from the six positive contacts and from six healthy controls and six tuberculous patients. ▥, healthy controls from endemic areas; ▤, tuberculosis patients; ●—●, contacts.

The age and sex distribution of the patients studied is shown in Table 1. Two of the 54 (4%) contacts under 15 years old were antibody positive and 4 of 46 (9%) contacts of 15 years and over were antibody positive. The early lepromin reaction was read in 91 cases and 21 of these returned after one month for reading of the late reaction. Nine cases were lost to follow up. Of the 6 SACT positive contacts 4 had a negative lepromin test (all Fernandez reaction) and 2 a positive test (one Fernandez reaction and one Mitsuda reaction) (Table 1). The duration that each contact was exposed to the source case is shown in Table 2. Only one out of the 31 (3%) contacts exposed to LL cases was antibody positive whereas 4 out of 37 (11%) contacts of BT cases were antibody positive. There was thus no positive correlation between the antibody positivity of contacts and exposure to a multibacillary source case. Only 2 out of the 24 (8%) contacts exposed to a source case for 10 or more years was antibody positive whereas 4 of the 76 (5%) contacts exposed for less than 10 years and 2 of the 42 (5%) exposed for less than 5 years

Table 1. Age/Sex distribution and lepromin status of contacts

Age (years)	Number of contacts			
	Sex		Lepromin	
	male	female	positive	negative
0–4	6*	9	4	10*
5–9	16*	4	7	13*
10–14	14	5	9	10
15–19	7	3*	7	1*
20–24	6	3	3	5
25–29	4	1	0	3
30–34	2	3	3	2
35–39	4*	1	2*	3
40–44	2	2*	3*	1
45–49	2	2	2	0
50+	3*	1	0	3*
Total	66	34	40	51

* One SACT positive contact

Table 2. Survey of the duration of exposure of contacts & classification of source cases

Duration of exposure (years)	Number of tested contacts				
	TT†	BT	BB	BL	LL
0–4	2	20*	7	6	7*
5–9	—	10**	5	7	12
10–14	—	3	1	3*	8
15–19	—	4*	—	—	1
20–24	—	—	—	1	3

* One SACT positive contact
† Classification of the source case.

were antibody positive. There was thus no positive correlation between antibody positivity and duration of exposure to a source case.

Discussion

Only a small proportion of those exposed to leprosy will go on to develop disease:

the incidence of leprosy in a marriage partner of a patient is in the order of 5%.[8] It is therefore impractical to follow up and/or prophylactically treat all contacts of leprosy patients. However, preventative treatment could be considered if a small high-risk group could be identified. Only 6 out of 100 household contacts of leprosy patients were SACT positive. Antibodies detected by the fluorescence test were observed in a much larger proportion of contacts: one study[9] found them in 81% (21 out of 26) in a similar local population to that of this study and another[1] found them in 92% (57 out of 62) of contacts in Japan. The MAB competition test thus defines a much smaller sub-group of all those with a subclinical infection.

Assuming that antibody positive contacts have a higher risk of subsequently developing leprosy than antibody negative contacts, this small fraction of all contacts could be carefully followed up. In this study 4 of the 6 antibody positive contacts had a negative lepromin test. It is accepted that cell-mediated immunity rather than antibodies can eliminate a subclinical infection.[3] Thus the antibody positive contacts who lack a cell-mediated immune response to *M. leprae* as judged by a negative lepromin test would seem to carry the highest risk and could be considered for chemoprophylaxis.

It has been generally assumed that contacts exposed for prolonged periods to a multibacillary case and childhood contacts probably carry the highest risk. Although our study contained only 6 antibody positive contacts the obtained results did not support such trends. Two of the antibody positive contacts were relatives, husband and son, of a 39-year-old paucibacillary case (BT) and they had only been exposed to the overt disease for 3 years. The possibility remains that these two contacts were also exposed to another source as they lived in an area of moderate endemicity; the leprosy prevalence rate in the catchment area of this study is 8 per 1000 head of population. Childhood contacts were not more likely to be SACT positive than adults: 4% (2 out of 54) of those under 15 and 6% (1 out of 15) of those under 5 were seropositive. One study[1] found fluorescent antibodies in higher titres in those under 5 years of age; the only positive contact under 5 in our study had a low titre of antibody as had all the other seropositive contacts.

In the previous study[5] a higher overall antibody positivity (30%) was reported among the contacts of leprosy patients. However, no information about the age and sex of these contacts as well as the endemicity of the area to which they belonged was recorded. It is possible that most of those contacts belonged to hyperendemic areas and were an older group with prolonged contact with the source cases. In the present study, only 9% positivity among the contacts above 15 years and 4% in those below 15 years of age was found.

Only a longitudinal prospective study could define the significance of antibody positivity by assessing the clinical outcome in the positive contacts and evaluating the predictive value of the MAB competition test. Preliminary results from a prospective study[10] indicated that 6 out of 16 SACT positive contacts developed overt leprosy manifesting as multiple lesions of the BT/BB type within

6 months of the serum being positive. Some of the contacts who were initially SACT positive became negative and developed a positive Mitsuda reaction. This ongoing survey suggests that those contacts who are ML04–SACT positive may be at a greater risk of developing clinical leprosy.

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