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IgM IMMUNOGLOBULINS REACTING WITH THE PHENOLIC GLYCOLIPID-1 ANTIGEN FROM *MYCOBACTERIUM LEPRAE* IN SERA OF LEPROSY PATIENTS AND THEIR CONTACTS

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For the first time in Brazil it was investigated the occurrence of IgM anti-PGL-1 in the sera of household contacts of leprozy patients using the ELISA methodology. The sera of the multibacillary patients showed significantly more immuno-reactivity than from the paucibacillary patients. It was observed a high subclinical infection incidence among household contacts (19.4%). The percentage of leprosy development was 5% (1/21) among the seropositive contact group. This finding suggests that serology could be useful as prognostic test, but for better definition is necessary to test a population from endemic area for long period time.

Key words: ELISA – IgM anti-PGL-1 – household contacts

The discovery and the elucidation of the chemical structure of a specific phenoglycolipid of *Mycobacterium leprae* (Hunter & Brennan, 1981) and the finding that it was antigenic (Hunter et al., 1982; Payne et al., 1982; Young & Buchanan, 1983) was a major breakthrough in leprosy research. The purified antigen (PGL-1) was used in many studies and is showed that leprosy patients in the lepromatous side of the spectrum formed IgM immunoglobulins reacting against this antigen, while most patients in the tuberculoid side of the spectrum did not form the specific immunoglobulins (Cho et al., 1983; Young et al., 1984). Other investigators showed that during chemotherapy there was a decrease in serum antibodies (Brett et al., 1983; Bach et al., 1986) or a decrease in the concentration of the PGL-1 in the tissues (Young et al., 1985; Cho et al., 1986).

It was thought that the serological methods using the PGL-1 could also be useful in identifing among close contacts of leprosy patients those infected by *M. leprae* (Douglas & Worth, 1983; Chanteau et al., 1987). As data on this important question remains limited, we decided to investigate the occurrence of IgM immunoglobulins in the sera of close contacts of leprosy patients. Also, because the PGL-1 antigen was not evaluated before in Brazil we assayed the sera of leprosy patients, who were the index case of the contacts selected for this study.

MATERIALS AND METHODS

Population – Three-hundred-fifty seven subjects living in Rio de Janeiro were selected for this study and divided as following: 131 Leprosy patients (74 multibacillary – 42 LL and 32 BL – and 52 paucibacillary patients – 2 BB; 21 BT and 29 TT); 108 household contacts (C); 28 professional contacts (Co) among the staff and laboratory workers (FIOCRUZ) and 92 negative controls (52 healthy individuals –N and 40 tuberculosis patients –Tb). The age of the household contacts ranged from 8 to 70 years (average age: 30 years) 38.6% males and 60.8% females, all of them resided with the index case.

All leprosy patients were classified according to Ridley & Jopling (1966) by Dr Almeida, S. M. R. – Leprosy laboratory – FIOCRUZ. Among the leprosy patients, 30 were untreated.

Sera – The sera from patients, contacts (Souza Araújo Ambulatory – FIOCRUZ) and control were stored at –4 °C with glycerol (v/v) until ready to use.

Microplate ELISA (Enzyme-linked immunosorbent assay) – A modified procedure described

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TABLE I

Human IgM serumreactivity to PGL-1

Population	ELISA			
	No All case	No Positive	(%) Positive	Optical density Means (X ± SD)[a]
LL	42	35	(83.3)	0,84 ± 0,50
BL	32	26	(81.3)	0,81 ± 0,52
BB, BT/TT	52	06	(11.5)	0,16 ± 0,11
C	108	21	(19.4)	0,17 ± 0,20
CO	28	03	(0.7)	0,15 ± 0,12
N	52	04	(7.7)	0,11 ± 0,08
TB	40	02	(5.0)	0,11 ± 0,08

LL – Lepromatous Leprosy; BL – Borderline Lepromatous; BB – Borderline Borderline; BT – Borderline Tuberculoid; TT – Tuberculoid Leprosy; C – Contacts; CO – Professional contact; N – Healthy Individual; TB – Tuberculosis Patients.

[a] Means ± standard desviation.

by Cho et al. (1983) was applied. Polystyrene microtitre plate (Dynatech Lab.) with flat bottom and 96 wells (Immunol 2) were coated with 100 μl of PGL-1 suspension (2 μg/ml) diluted in ammonium acetate buffer, pH 8.2, sonicated for 30 to 60 s with a 9 mm probe (Blackstone Inc.), and incubated at 37 °C overnight. Wells were washed with phosphate-buffered saline (PBS), blocked by the addition of 200 μl of PBS containing 5% bovine serum albumin (BSA), and incubated at 37 °C for 1 h in a moist chamber. After washing, 100 μl of the conjugate (goat anti-human IgM-peroxidase, Organon-Cappel Lab.) diluted 1/4.000 in PBS-BSA 1%. After 1 h incubation, the plates were washed, 100 μl of H_2O_2-O-phenylediamine substrate-dye reagent in citrate phosphate buffer (Abbott Lab. of Brazil Ltd.) were added and incubated for 20 min at room temperature in dark. Reactions were terminated with 4N H_2SO_4. The plates were read in a "Titertek Multiscan" spectrophotometer (Flow Lab.), at 492 nm.. The PGL-1 results were expressed as optical density variation (Δ OD ratio of OD of the test serum from coated plates minus those from the uncoated plates). The serum was considered positive when the OD exceded by two standard deviations (SD) the mean of results from healthy controls (XN + 2 SD).

For control tests were assayed in parallel antigen blank wells to ensure that positive reading were, in fact, due to specific interations between antibodies and the antigen.

Each serum was tested in duplicate. In each set of experiments a reference positive serum and negative serum were included.

Clinical follow-up – All contacts were clinically observed over a 2 year period for the evidence of the development of leprosy.

RESULTS AND DISCUSSION

Preliminary observations – The distribution of ELISA results in the sera of the populations studied is showed in Table I. The sera from multibacillary patients (LL, BL) was significantly more immunoreactive than in paucibacillary patients (BB, BT/TT). The percentual reactivity among the several clinical forms of leprosy is showed in Table I. The 131 leprosy patients tested presented serum positivity of the: 35/42 (83.3%) LL, 26/32 (81.3%) BL, and 6/52 (11.5%) BB, BT/TT. The high positivity observed in multibacillary patients were expected (Brett et al., 1983; Cho et al., 1983). The low percentage of serum positivity among the paucibacillary patients may be reflect the high "cut off" used in this study (0,27 = mean + 2. SD of the healthy control group).

In contrast with the data of others that found out few inespecific reaction among healthy individuals and tuberculosis patients, (Anonymous, 1986; Brett et al., 1986) (around 3% of false positive reactions) our results presented the most reactive rate with these groups (6.5%) (Table I). This is likely due to

the high prevalence rate for tuberculosis and leprosy in Brazil (0.8% and 1.7% respectively). We also observed that sera from malaria and leishmaniose patients cross reacted with PGL-1. These data bring restrictive implications for the use of the test in area where these diseases show a high prevalence (data not showed).

The distribution of the household contacts in respect to the clinical form of the index case are showed in Table II. The IgM antibody were detected in the sera of 21 contacts (Table III). The frequency of positive serology among these contacts was therefore 19.4%. The ages of these contacts ranged from 6 to 61 years (average age: 25 years). Among the 28 professionals contacts attending leprosy patients, 3 had positive serology (10.7%) (Table I). Most of these subjects was lepromin positive, only 5 showed a lepromin negative test.

TABLE II

Characteristics of the household contacts

Index case	Household contacts: Age < 15 years		> 15 years	
	Female (%)	Male (%)	Female (%)	Male (%)
LL	(0.9)	(0)	(12)	(5.5)
BL	(11)	(12)	(32.4)	(16.6)
BB	(0)	(0)	(2.7)	(1.8)
BT	(0)	(0)	(1.8)	(2.7)
TT	(0)	(0)	(0)	(0)
Total	(11.9)	(12)	(38.9)	(26.6)

The clinical examination of the 108 household contact revealed only one (0.9%) case of leprosy after 2 years of following up, and this contact was a 20 years old man with a Δ OD 0,83 ELISA test. One year later he developed intermediate leprosy, with a Mitsuda negative test.

None of the professional contacts developed leprosy during the study.

The results pointed out that the percentage of leprosy development is greater among the contact of leprosy patients than in the professional and normal controls. However the only case of leprosy occurred in the seropositive contact group, increasing the development rate of leprosy to 5% (1/21) (Table III).

TABLE III

Rate of leprosy in the household contacts after two years

	Number tested	Leprosy case (%)
Serum positive group	21	1 (5)
Serum negative group	87	0 (0)
Total	108	1 (0.9)

For a more precise definition whether the ELISA will be useful or not as a prognostic test it is necessary to test a population from endemic area at least up a 5 year period. Then, this population should be followed up with careful clinical examination and periodic IgM antibodies anti-PGL-1 detection.

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