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Seroreactivity against the *Mycobacterium leprae* phenolic glycolipid I in Mycobacteria infected or stimulated groups of individuals

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Summary The enzyme-linked immunosorbent assay (ELISA), was applied in a group of sera of lepromatous leprosy patients, tuberculosis patients, BCG vaccinated children and bloodbank donors using the phenolic glycolipid I, isolated by Hunter and Brennan, for the determination of specific antibodies.

Positive results were found in the group of leprosy patients while the majority of the sera of the other individuals were negative.

Slight cross-reactivity was encountered in a few individuals.

At the same time a study was carried out in healthy persons without a known contact with *Mycobacterium leprae*. These received a lepromin injection (Mitsuda test) and blood samples were taken before and 21, 45 and 90 days afterwards. In this case evidence was shown that the lepromin injection did not influence the results of the test.

Introduction

During the immune reaction to *Mycobacterium leprae* infection, humoral response which is generally accepted as non-protective, arises. This characteristic may be of importance to analyze the variable course of infection, the immunogenic structure of the bacilli and for the detection of subclinical infection.[4,8]

Harboe *et al.* have suggested that if antibodies arise after infection and before symptoms appear it may be possible to study the epidemiology of leprosy infection instead of the epidemiology of the disease and its complications as has been done previously.

Several investigators[3–5,10] have shown that antibody concentration is higher in multibacillary leprosy than in the paucibacillary type which may relate antigenic load to antibody titres. This relationship might be important for the early

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diagnosis and control of the individuals responsible for the transmission of the disease in the community.

Recently, Hunter and Brennan[6] obtained an antigen which is considered species specific for *M. leprae* and might be useful for the serological screening in high risk population by the ELISA test.[2,6] Since the phenolic glycolipid I has been made available to us by the courtesy of Dr P Brennan, we have examined its activity against sera from lepromatous leprosy patients, tuberculosis patients, BCG vaccinated children, lepromin tested healthy volunteers and bloodbank donors.

Materials and methods

Sera of 32 lepromatous clinically and histologically classified patients were included in this study. Of these, 9 were under DDS treatment. Also the sera of 27 tuberculosis patients, 24 BCG vaccinated children and 195 bloodbank donors were studied.

Simultaneously 33 healthy Cuban volunteers, without known contact with leprosy patients, received a lepromin injection (0·1 ml/40×10^6 bacilli/ml Lepromin A from Carville USA). Blood samples were taken just before the injection and 21, 45 and 90 days thereafter and their sera tested by the ELISA technique.

ELISA conditions:[1] The phenolic glycolipid I antigen was suspended in ethanol to a concentration of 2 μg/ml and 50 μl added to wells of polysterene plates which were incubated overnight at room temperature. Plates were washed with phosphate buffered saline (PBS), blocked with PBS containing 5% bovine serum albumin (PBS/BSA) and incubated at 37°C for 1 h in a moist chamber, after which the PBS/BSA was aspirated. Fifty microlitres of serum diluted 1:300 with PBS containing 20% normal calf serum (PBS/NCS) was added and incubated at 37°C for 1 h. Later, plates were washed with PBS and goat antihuman immunoglobulin M (IgM) peroxidase conjugate (Cappel Laboratories) diluted 1:1000 in PBS/NCS was added. After a 1 h incubation the plates were washed and 50 μl of H_2O_2–Ophenylendiamine substrate in citrate-phosphate buffer were added and incubated at room temperature for 20 min in the dark. The reaction was stopped with 2·5N H_2SO_4 and the absorbance read at 492 nm using a Multiskan MC reader (Flow Lab).

Reference sera, positive and negative were included in each plate to correct the sample readings.

Statistical analysis was carried out using the χ^2 test.

Results and discussion

Two-fold dilutions from 1:20 to 1:2560 of negative sera and 2 positive sera were

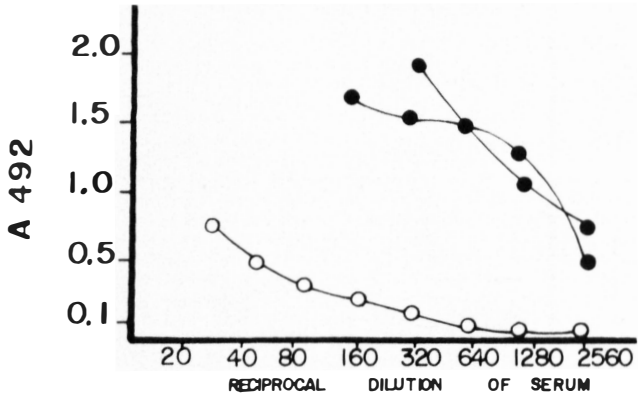

Figure 1. IgM activity against phenolic glycolipid in serial dilution of lepromatous sera (●——●), and negative human serum (○——○).

used to standardize the ELISA test (Figure 1). We chose 1:300 as working dilution since its combination with the antigen gave the maximal separation between positive and negative test samples but still showing low negative values. A total of 300 sera of bloodbank donors were analyzed and compared to negative human control sera to determine the cut-off value at $\bar{x} \pm 2$ SD ($0{\cdot}060 \pm 0{\cdot}124$). The study of 23 sera of untreated lepromatous patients demonstrated a high positivity (100%) using the phenolic glycolipid I in the ELISA test. These results agree with those encountered by other investigators[3–5,9] in which reference is made between bacillary load and antibody production, both of which are very high in patients presenting this clinical form of leprosy (Table 1 and Figure 2). The results obtained with 9 treated lepromatous patient sera, demonstrated a similar behaviour to that found by others[3–5,7,10] in patients with DDS treatment, who point out that antibody titres decrease in relation to the time of treatment. At the

Table 1. Seroreactivity to phenolic glycolipid I.

Sera	No. positive/negative	X ± SD (A492)	Positivity (%)
Multibaciary patients not treated	23/0	$1{\cdot}51 \pm 0{\cdot}655$	100
Multibaciary patients treated	7/2	$0{\cdot}903 \pm 0{\cdot}766$	77·7
Tuberculosis patients*	2/27	$0{\cdot}073 \pm 0{\cdot}073$	7·41
BCG vaccinated children*	1/23	$0{\cdot}088 \pm 0{\cdot}050$	4·34
Bloodbank*	10/185	$0{\cdot}170 \pm 0{\cdot}062$	5·12

* $\chi^2 = 0{\cdot}313$ $P > 0{\cdot}01$.

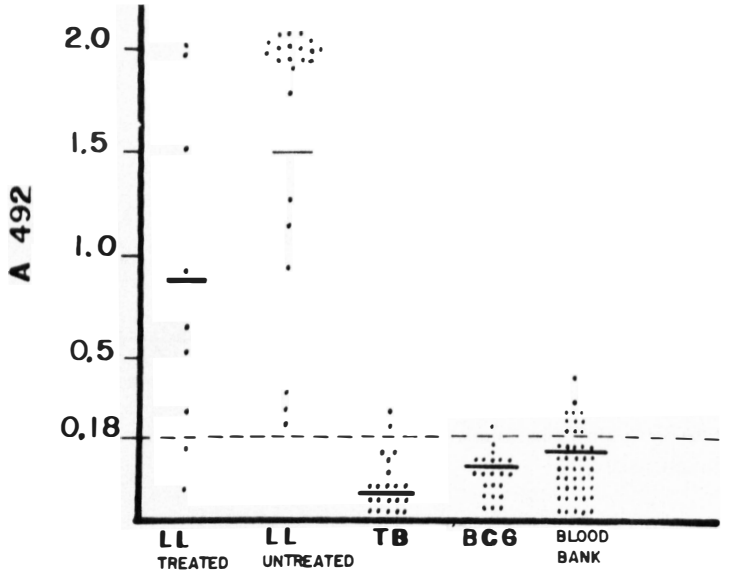

Figure 2. IgM antibody activities against Phen Gly I in human sera. The solid lines represent the mean values and all points above the dashed line are considered positive.

Table 2. Seroreactivity to phenolic glycolipid I in treated LL patients.

Sera	Bacteriological Index (BI)	Time of treatment (years)	OD
1	0	21	0·04
2	0	12	0·16
3	1	5	0·25
4	3	4	2·00
5	4	2	0·89
6	2	2	2·00
7	2	2	0·55
8	3	2	0·70
9	3	1	1·54

same time a tendency towards a reduction in the Bacteriological Index (BI) is observed although certain differences are seen among these patients (Table 2).

In the study of the group of tuberculosis patients and BCG vaccinated children the results in general are negative as can be observed in Table 1. Nonetheless, we cannot ignore a discrete positivity in both groups: 7·4% in the tuberculosis patients and 4·2% in the BCG vaccinated children.

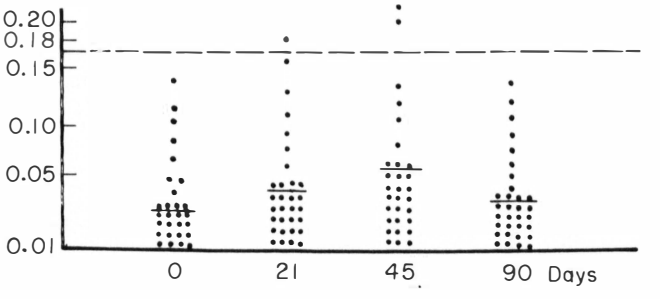

Figure 3. Lepromin influence in healthy people. The solid lines represent the mean values and all points above the dashed line are considered positive.

The sera of supposedly healthy bloodbank donors also demonstrated slight positivity of 5·1% which is comparable to the results reported by Young and Buchanan with healthy individuals in their work in Mexico (4%) and Sri Lanka (9%).[9]

The positive results of those individuals who do not present the disease, were analyzed by the χ^2 test which demonstrated that there was no significant difference ($P > 0{\cdot}01$) between them while a significant difference was found when compared to the leprosy patients ($P < 0{\cdot}01$). These positive results may be due to a discrete cross-reactivity, or as a result of contact with *M. leprae*, or instead, simply false positive reactions.

Among the persons who received a lepromin injection (Mitsuda test) (Figure 3), a slight positive result can be observed in one individual at 21 days and in two at 45 days, while all persons in the study were negative by 90 days. The negative results with the lepromin test seem to confirm the view about the poor immunogenicity of the phenolic glycolipid I[3] since the injection of 4×10^6 dead organisms was not enough to elicit a positive response.

Our results seem to confirm that ELISA using the phenolic glycolipid I is potentially a highly specific procedure for the detection of antibodies against leprosy infection without complications arising from BCG vaccination or previous infection with *M. tuberculosis* and encourage us to undertake a more extensive trial among contacts of leprosy patients aimed to detect early stages of the disease and to assess its predictive value.

Acknowledgments

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