

ANTI *M. leprae* IgM ANTIBODY DETERMINATION BY ULTRAMICROIMMUNOENZYMATIC (UMELISA HANSEN) FOR THE DIAGNOSIS AND MONITORING LEPROSY

Adriana TORRELLA (1), Rosa L. SOLIS (1), Esther PEREZ (1), Yadira MEDINA (2), Carlos KERGUELEN (3) & Patricia OLAYA (4).

SUMMARY

The relationship between the IgM antibody response, antigenic load as well as the clinical improvement after chemotherapy was studied in order to obtain useful data for the early diagnosis and monitoring leprosy. A level of 82% (94/115) agreement was obtained between IgM UMELISA HANSEN and slit-skin smear examination. Discrepant results were observed in 16 patients who showed positive IgM response despite negative by the skin smear examination. In these patients, the IgM response was seen to be associated to the early signal for bacilli recurrence in the skin. In one of these patients the presence of bacilli was demonstrated in the skin, two months after IgM antibodies being detected by UMELISA HANSEN. Also in one of the treated patients positive by both diagnostic techniques, a remarkable decrease in the IgM antibody levels was seen, correlating with a significant clinical improvement. Moreover it was found a direct relationship between the IgM antibody response and bacterial antigenic load, regardless the time elapsed in the disease's evolution.

KEYWORDS: UMELISA HANSEN; Leprosy; Phenolic Glycolipid-I.

INTRODUCTION

Leprosy is a chronic infectious disease and its diagnosis is still a subject of controversy, as the pathogeny is not well defined as well as the way of transmission remains doubtful. This infection has a long incubation period and its initial symptoms are not perceptible. Thus at the time when the clinical diagnosis is made, *Mycobacterium leprae* (*M. leprae*) has already been disseminated throughout the community.

So far, the leprosy is still a public health problem in many countries. Its gravity in relation with other diseases can not be precised because of factors as the number of unknown affected patients, duration of the disease, incapacities that it causes, and human and social consequences for patients and their families.

There are two polar forms of leprosy, the lepromatous form with deterioration of the cellular immunity, high antigenic load and antibodies in high levels, and the tuberculoid form, in turn, with intact parameters of cellular immunity, scarce bacilli and moderate or very low antibody levels. Thus, serologic techniques for the detection of antibodies to *M. leprae* are mainly designed for the diagnosis of lepromatous leprosy.

In this work, we refer to the potential fullness of an ultramicro ELISA that employs as antigen, a synthetic disaccharide of phenolic glycolipid-I (PGL-I) attached to bovine serum albumin (BSA), for the detection of IgM antibodies to *M. leprae*. The main objective is to investigate the relationship between the IgM antibody response and presence of bacilli in order to evaluate the UMELISA HANSEN not only in the search for preclinic cases, but also in monitoring leprosy patients, as well as its possible application in population surveys that would ultimately lead to the control of this disease.

MATERIALS AND METHODS

Samples: A total of 115 leprosy patients were studied: 88 of these from the Dermatological Specialized Hospital "Guillermo Fdez. Hdez.-Baquero" in Rincón, Havana, Cuba, and 27 patients from the Hospital at the University of Barranquilla, Colombia. These patients were classified according to the clinical criterion established in the VI International Congress of Leprosy, as follows: 96 with lepromatous (L), 12 with borderline lepromatous (dimorphous) (B) showing multibacillary forms, 3 with indeterminate (I) and 4 with tuberculoid (T) presenting paucibacillary forms. Eighty three patients of the 115 patients were in the surveillance period subsequent to the

(1) Immunoassay Center, C. Habana, Cuba.

(2) Dermatological Specialized Hospital "Guillermo Fdez.Hdez.-Baquero", Rincón, Havana, Cuba.

(3) University Hospital, Barranquilla, Colombia.

(4) BIOLEHNER, Bogotá, Colombia.

Correspondence to: Lic. Adriana Torrella. Centro de Inmunoensayo, Apart. 6945, Playa, C. Habana, Cuba.

multidrug therapy, and the remaining 32 patients were under chemotherapy at the moment of study. In the skin smear examination (below described), 19 patients were considered positive and 96 negative.

Serum samples were collected from above patients and also from 230 individuals at close contact with patients, comprising 220 workers of the Leprosy Center at Rincon, and 10 household contacts of the patients in Colombia, and were assayed in the UMELISA HANSEN.

Slit-skin smear examination: Four slit-skin smears, from both earlobes and at least two active sites, were used for serial determinations of the average bacterial index (BI) in each patient according to Ridley-Jopling scale¹⁰. If BI > 0 the slit-skin smears was considered positive and this finding was generally associated with the multibacillary forms of the disease. This technique was performed in the clinical laboratory of the Hospital prior to the serum evaluation by UMELISA HANSEN.

This microbiological technique was repeated only in patients showing positive IgM UMELISA HANSEN but not in those with negative IgM showing positive slit-skin smear.

UltramicroELISA HANSEN: This enzyme immunoassay was carried out as described¹³ previously. Briefly the samples diluted 1:40 in Tris-Tween, containing 5% of Sheep serum, were added in duplicate to the ultramicroplates coated with a synthetic disaccharide of PGL-I (antigen) and incubated at 37°C. Ultramicroplates were washed four times with Tris 0.15 M pH 7.8 and sheep anti-human IgM alkaline phosphatase conjugate, diluted 1:8000, was added. Ultramicroplates were washed again, and the substrate solution was added (4-Methylumbelliferyl phosphate dissolved in diethanolamine buffer). The fluorescence was measured with SUMA reader at 480 nm. The samples were considered positive when their fluorescence intensity in relation to the positive serum control were greater than or equal to 0.3. There are three ranges of positivity established for the assay, according to the classification of the Leprosy Control Cuban Program: 0.300 to 0.399 = Doubtful, 0.400 to 0.499 = Suspect, and > 0.499 = High.

RESULTS

A high level of agreement, 82% (94/115) was obtained between the UMELISA HANSEN and slit-skin smear technique.

A total of 21 patients showed discrepant results, 16 of them were seropositive but with negative slit-skin smear (BI = 0), and 12 out of these 16 patients were in the surveillance period without chemotherapy for a year or more.

Conversely, only 5 cases showed no detectable IgM antibody but were positive by slit-skin smear (BI > 0).

In Fig. 1B, the highest frequency of IgM seropositivity is shown in the patients with positive slit skin smear (BI > 0). The

seropositivity was 74% (14/19) for this group whereas in the group of patients with negative slit-skin smear (BI = 0) (Fig. 1A) the seropositivity was only 17% (16/96) with highest frequency (83%) of negative serology.

Irrespective the evolution time elapsed, significant IgM levels remain in leprosy whenever bacilli are demonstrated in patients (Table 1). A Spearman correlation coefficient¹² (r_s) of 0.83 was obtained between the bacterial index and the UMELISA HANSEN.

In the contact population, the seropositivity was 3.5% (8/230), but presented low levels of IgM.

Sixteen discrepant cases were positive by UMELISA HANSEN and negative by slit-skin smear, and 13 of these had lepromatous leprosy, two had borderline lepromatous leprosy and one had tuberculoid leprosy. Moreover in the group of 14 IgM seropositive patients who presented clinically active disease, high levels of IgM antibodies were detected in most of the patients with multibacillary forms of the disease.

In the group of positive patients for both UMELISA HANSEN and skin smear, and received treatment, there was a patient who showed a marked clinical improvement, three months after these laboratory examinations. Thus, the clinicians suggested to repeat both assays for therapeutic control. It was disclosed that the decrease of antibody levels from high to doubtful and finally to BI = 0 correlated positively to the clinical improvement in this patient.

In patients at the surveillance period, high levels of anti PGL-I IgM response were measured sequentially but the microbiological technique was repeatedly negative, demonstrating the persistence of antibody response. In one of the patients however, bacilli were found in the skin (BI > 0) two months after the last examinations which had given positive IgM and negative slit-skin smear. The rest of the patients remains under observation because it has been reported² that the relapse may occur at least, two years after treatment.

DISCUSSION

In the present work we do not investigate the UMELISA HANSEN taking the slit skin smear as a gold standard because these techniques are based on different analytical principles, and also by the fact that the immunoenzymatic techniques are highly sensitive⁷. Therefore, we only assessed the degree of agreement between the IgM response and the presence of bacilli in the skin, and also the feature of the immune response as a marker for the diagnosis and monitoring the treatment of leprosy.

Taking all the results together, we understand that the agreement obtained between both techniques is good, though discrepancies were observed in 16 cases in which IgM was detected without clinical symptoms of the illness. The great majority of the evaluated population did not receive treatment

TABLE 1
Immunoglobulin M antibodies to PGL-I and bacterial index in patients with leprosy.

Evolution Time	Bacterial Index	UMELISA Results	Classification for UMELISA
2 months	0.6	0.35	DOUBTFUL
5 months	2.8	0.96	HIGH
5 months	2.2	0.88	HIGH
6 months	2.0	0.81	HIGH
1 year	2.6	1.20	HIGH
1 year	2.6	0.80	HIGH
1 year	2.4	0.89	HIGH
1 year	0.4	0.42	SUSPECT
1 year	0.2	0.42	SUSPECT
2 years	0.8	0.59	HIGH
3 years	2.2	0.67	HIGH
3 years	1.2	0.53	HIGH
4 years	2.4	0.55	HIGH
20 years	2.0	0.57	HIGH

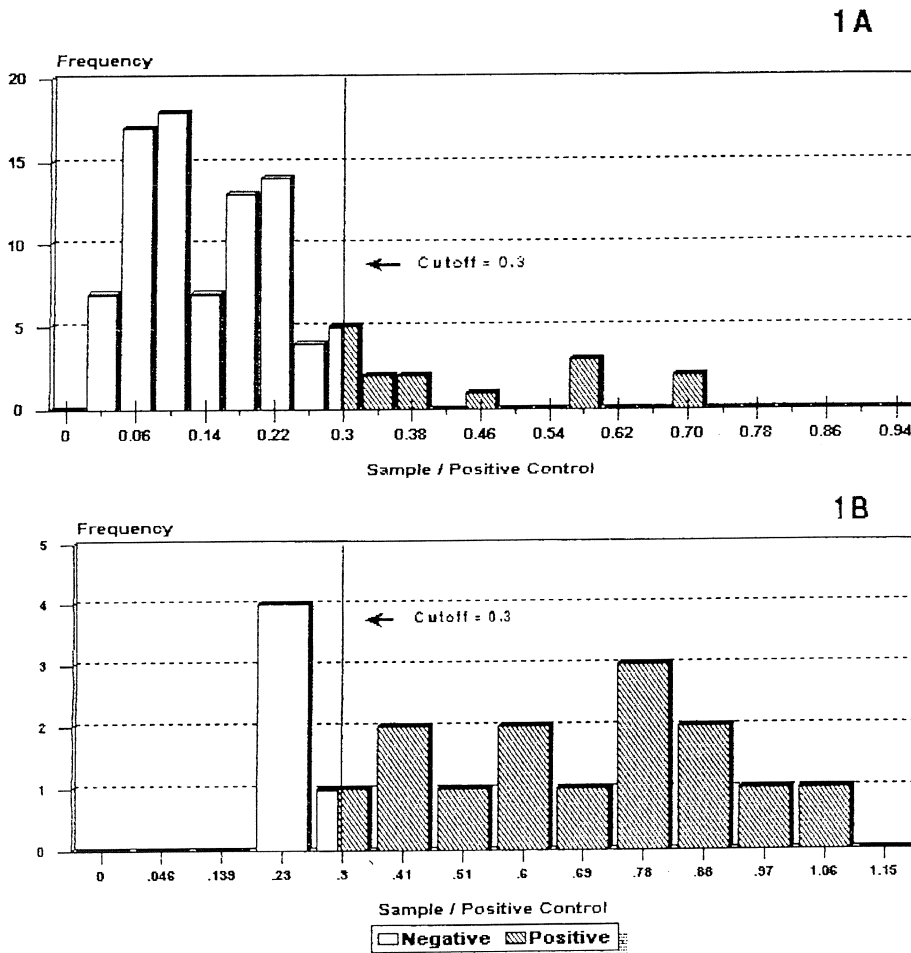


Fig. 1 - Distribution of the anti PGL-I IgM response in relation with B.I. 1A: Patients with B.I.=0, 1B: Patients with B.I.>0.

for more than a year and it is possible that high levels of IgM antibodies are associated to the beginning of a relapse although bacilli have not been detected yet in the skin.

It is relevant to emphasize that most of the patients, who gave positive IgM and negative slit-skin smear, were already considered negative by the persistence of negative slit-skin smear after treatment. These data support the usefulness of the assay for IgM detection during the surveillance period to monitor leprosy patients, once they were considered negative, particularly in those who had a multibacillary form.

It would be recommendable to repeat the bacteriological test to those five patients who showed negative UMELISA HANSEN and positive slit-skin smear because they did not receive chemotherapeutic treatment and their slit-skin smear results were not recent.

Probably bacilli have been eliminated, but this has to be checked, as in immunocompromised patients bacilli may be found frequently in the absence of antibodies.

The highest frequency of positive IgM response in patients with positive slit-skin smear and the persistence of this antibody while bacilli are present, regardless the disease's evolution time, as well as the high correlation obtained ($r_s=0.83$) between IgM response and bacterial index, are supported by the reported data^{1,3,11}. The elevated IgM anti PGL-I levels in direct relationship with the antigenic load is indicative of an active infection, showing that IgM detection is useful for the diagnosis and monitoring leprosy.

The low seropositivity observed in the group of individuals who have a close contact with patients is expected because the transmission of the disease was prevented by treating all patients with leprosy. It has been reported⁶ that due to the high sensibility of the immunoenzymatic assays, although they are mainly designed for the diagnosis of lepromatous leprosy, it is possible the detection of other forms of the disease.

Thus the UMELISA HANSEN corroborates this judgment, presenting sensitive results and allowing diagnosing not only the lepromatous leprosy but also some other forms of the disease such as, dimorphous and tuberculoid. It is also observed the association between the high IgM levels and the multibacillary forms of the disease.

The decrease of bacilli correlates with the effectiveness of the chemotherapy and it was seen a relationship between the decrease of bacilli and the decrease of IgM response in one patient. This finding points out that the IgM response may be a good parameter to measure the efficiency of the adopted chemotherapy⁸.

The detection of bacilli, in one patient who had presented initially high IgM levels but negative slit-skin smear, provides additional support to our view point as well as of the others^{5,8,9} as

to the value of IgM response when clinical symptoms are not evident. This antibody isotype shows to be an immunologic marker, for early diagnosis of leprosy and seems also suitable for the screening of those with recurrent disease in the surveillance period.

The data demonstrate that the detection of IgM Anti PGL- I by the UMELISA HANSEN may contribute to the early diagnosis and to monitor leprosy during the chemotherapeutic treatment as well as in the surveillance period. The features of this assay show its potential application to better control the disease, and consequently solving many of connected human and social problems.

The UMELISA HANSEN presents advantages offered by the SUMA technology, requiring only 10 μ l serum samples and reagents and permits to carry out easily population surveys in the public health laboratory engaged to the program of leprosy prevention and control.

RESUMEN

El UMELISA HANSEN en el diagnóstico y seguimiento de la lepra.

Se analizó la relación entre la carga antigénica y la respuesta de anticuerpos IgM, el comportamiento de dicha respuesta y la utilidad de su detección para el diagnóstico y seguimiento de la lepra. Se obtuvo un 82% de coincidencia entre los resultados del UMELISA HANSEN y los de la baciloscopía. Este valor se vio afectado fundamentalmente por 16 pacientes con respuesta positiva a IgM y baciloscopía negativa. En estos pacientes de acuerdo a lo reportado, la respuesta IgM puede indicar la reaparición de bacilos, precediendo a su detección en la piel. En uno de estos pacientes se demostró la presencia de bacilos, dos meses después de resultar positivo por el UMELISA HANSEN. Entre los pacientes coincidentes en IgM y baciloscopía positivas con tratamiento quimioterapéutico, se apreció en uno de ellos una notable disminución en los niveles de IgM en correspondencia con una mejoría clínica. Además se observó una conservación de la respuesta de anticuerpos IgM en relación directa con la carga antigénica, independientemente del tiempo de evolución de la enfermedad.

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