Anti-phenolic glycolipid 1 IgM antibodies in leprosy patients and in their household contacts

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Summary Though they have no apparent protective action, the specific antibodies are important markers of the infection with *Mycobacterium leprae*. For their detection we employed an ELISA method using as substrate a synthetic immunodominant disaccharide of phenolic glycolipid 1 antigen of *M. leprae*, conjugated with bovine serum albumin (D-BSA). Increased levels of anti-D-BSA antibodies of the IgM class were detected in 61.5% of the 13 leprosy patients and in 13.3% of their 53 household contacts, whereas they were not found in any of the 37 normal blood donors. A strong correlation ($r = -0.846$) was found between the antibody levels and the duration of the disease among the 12 patients with lepromatous leprosy.

These preliminary data demonstrate the usefulness of this method for epidemiological studies and for the detection of cases with subclinical infection.

Introduction

The better understanding of the immune response to *Mycobacterium leprae*, the causative agent of leprosy, is very important not only in view of the production of a possible vaccine but also for the development of more sensitive serological tests.¹

The antigenic structure of *M. leprae* has been extensively studied in the last years and a phenolic glycolipid, called the phenolic glycolipid 1 (PGL-1), has been characterized to be one of the major antigens of this microorganism.² The unique trisaccharide portion of this antigen, containing an immunodominant terminal 3.6-di-methylglucose residue has been found to elicit both a B cell response and a T cell suppression *in vitro*.³

A sensitive enzyme immunoassay has been recently developed that detects specific antibodies to the PGL-1 antigen⁴ and this test has been used for epidemiological studies in different countries.⁵ We applied a similar study in Albania and our preliminary data have confirmed the usefulness of this method for epidemiological purposes revealing on the other part a strong negative correlation between the antibody levels and the duration of the disease among the patients with lepromatous leprosy.

Materials and methods

PATIENTS

103 individuals, all from Albania, have been studied for anti-PGL-1 IgM antibodies, 58 were male and 45 female, aged from 5 to 78 years.

Among these were 12 with lepromatous leprosy (LL), 1 with tuberculoid leprosy (TL) and 53 were their close household contacts, 37 healthy blood donors were taken as a control group.

The duration of the disease in all the leprosy patients from the time of the diagnosis, until the moment of blood collection for anti-PGL-1 antibody determination varied from 1 to 33 years, mean 19.9 years (Table 1).

In all the patients treated before 1981, the treatment was started with DDS and CIBA 1906 and since 1981 multidrug therapy (MDT) was introduced, composed of rifampicin, DDS and clofazimine. This therapy has been continued for at least two years, and thereafter, every two years, the patients have received MDT for 1–3 months for the prevention of relapses. At the time of the study all the patients were negative for *M. leprae* in skin biopsies

The index cases of all the household contacts were either their first degree relatives or their consorts with whom they had been living in close contact for many years.

METHODS

Anti-PGL-1 antibodies of IgM isotype were determined using an indirect enzyme-linked immunoassay (ELISA). For antigen we used a synthetic disaccharide that is the immunodominant epitope of PGL-1. The disaccharide was conjugated with bovine serum albumin (D-BSA). The unconjugated BSA was used in parallel as a negative control. The antigens and the negative and positive reference sera were kindly provided by Dr H D Engers (WHO Immunology Research and Training Centre, Geneva, Switzerland).

Table 1. Leprosy patients studied for anti-PGL-1 IgM antibody levels

Patient No.	Age	Duration of disease (years)	Type of leprosy	ELISA results (%)
1	62	33	LL	11.6
2	47	20	LL	10.6
3	50	22	LL	7.6
4	65	15	LL	100.0
5	73	23	LL	9.6
6	58	32	LL	8.3
7	38	24	LL	32.5
8	48	24	LL	28.4
9	46	9	LL	162.9
10	36	18	LL	93.7
11	42	24	LL	28.9
12	78	14	LL	123.0
13	47	1	TL	25.5

The polystyrene plates were coated for 14–18 hr at 37°C with either D-BSA or BSA diluted in pH 9.6 0.1 M carbonate-bicarbonate buffer. After saturating with PBS-Tween-1% BSA for 1 hr at 37°C and washing, 100 microlitre of a 1/300 serum dilution were incubated in quadruplicate for 1 hr at 37°C. The IgM antibodies were detected using a peroxidase-labelled goat anti-human μ chain (Institute Pasteur, Paris) and revealed with orthophenylenediamine in citrate buffer pH 5.1 and in presence of H₂O₂. The reaction was stopped with H₂SO₄ and the optic densities were read on a Titertek Multiscan at 492 nm.

All the sera were run in parallel with the conjugated antigen and as well with BSA alone, and the differences of the two results were taken into consideration. The absorbances obtained with 1/300 dilution of test sera were converted and expressed in percentage in relation to the same dilution of the positive reference serum that was placed on the same plates.⁶ Those sera which gave an optical density higher than 20% of the reference serum were considered to be positive.

The differences between the antibody levels of different groups were analysed using Student's *t*-test after logarithmic transformation of data. *R* coefficient was calculated for linear regression.

Results

A graphic representation of absorbances obtained with serial dilutions of a positive and a negative reference serum is shown in Figure 1. These curves and others obtained with different positive sera (data not shown) allowed us to choose a dilution of 1/300 for the anti PGL-1 IgM antibody detection since this dilution was generally found in the linear portion of the curves.

Raised levels of anti-PGL-1 IgM antibodies were observed in 61.5% of the leprosy patients, in 13.3% of their household contacts and in no one of the normal blood donors (Figure 2). The geometric means of the antibody levels in the three groups were respectively 49.4 ± 52.0 , 7.7 ± 9.5 and 2.0 ± 3.1 . A significant difference ($P < 0.1$) was found between the patient's group and the group of healthy contacts, and on the other side between the last group and the normal blood donors ($P < 0.01$).

The seven household contacts with raised levels of anti-PGL-1 IgM antibodies were found in four families where the index cases also resulted with high levels of these antibodies (Table 2). The duration time of the disease among these index cases varied from 1 to 14 years, mean 8.5 years, that is lower than the mean duration time of the disease (19.3 years) of all the leprosy patients.

We also studied the correlation between anti-PGL-1 IgM antibody levels and the duration of the disease among the 12 patients with lepromatous leprosy (Figure 3) and a strong correlation ($r = -0.846$; $P < 0.005$) was found between these two parameters.

Discussion

The ELISA method that we applied for the detection of antiphenolic glycolipid 1 IgM antibodies enters in the last generation of the serological tests used for the study of the *M. leprae* infections.³ The antigen used is a synthetic disaccharide which constitutes the antigenic determinant of the phenolic glycolipid 1, one of the principal antigens of

Figure 1. Titration curves of a positive S(+) and a negative S(-) reference serum.

Figure 2. Anti-phenolic glycolipid I IgM antibody levels in the 3 groups of individuals studied. The data are presented in percentages in relation to the positive serum of reference estimated as 100%.

Table 2. The household contacts with raised levels of anti-PGL-1 IgM antibody and their respective index cases

Contacts				Index Cases			
Nr	Age	ELISA results (%)	ELISA results (%)	Diagnosis	Family relation with the contact	Duration of disease (years)	Patient No.
1	36	66.4	162.9	LL	husband	9	9
2	8	27.6	162.9	LL	father	9	9
3	32	49.5	—	LL	father	10	deceased
4	14	32.2	—	LL	mother	10	deceased
5	12	30.4	25.0	TL	father	1	13
6	9	65.7	25.0	TL	father	1	13
7	20	66.8	123.0	LL	father	14	12

Figure 3. The correlation between anti-phenolic glycolipid I IgM antibody levels and the duration of the disease in the lepromatous patients.

M. leprae.⁷⁻⁹ Its specific antibodies are mainly of the IgM class and are specific markers for this infection.¹⁰ The specificity of this method is confirmed by the fact that we did not find detectable antibody levels in any of the normal blood donors.

The seropositivity of 61.5% in our patients that were all under treatment is comparable with the data of other authors that have found a seropositivity of 68% in

treated patients.⁵ Among the household contacts the seropositivity was 13·3%. These data are lower than the frequencies of 25–35% reported from other authors in studies performed in hyperendemic areas.¹¹ This may be due to the fact that in our study, all the index cases were under long term treatment and with negative results for *M. leprae* from skin biopsies at the time of the study. On the other part in hyperendemic areas the household contacts may have multiple contacts with leprosy in addition to the identified index cases, whereas in Albania where the prevalence of leprosy is rather low, with a rate of 0·016 per thousand in 1988,¹² the identified index cases are probably the only source of infection.

We found a strong correlation between the antibody levels and the duration of the disease among the patients with lepromatous leprosy. This may reflect the antigen load of the patients and the effect of chemotherapy since a decrease in antibody levels during treatment has been reported.⁵ This is also supported by our finding of raised levels of anti-PGL-1 antibodies only among those contacts whose index cases resulted with high antibody levels and with a mean duration of the disease that was much lower than the mean duration of all leprosy patients.

Our data support the usefulness of this method for epidemiological studies and for the detection of cases with subclinical infections.

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