INTERNATIONAL JOURNAL OF LEPROSY Volume 54, Number 2
Printed in the U.S.A.

Antibodies to Phenolic Glycolipid-1 and to Whole *Mycobacterium leprae* in Leprosy Patients: Evolution During Therapy[1]

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Antibody production, which does not play any protective role in leprosy, varies along the leprosy spectrum as a consequence of the graded bacillary load from the tuberculoid to the lepromatous pole. Since antibodies represent an early sign of antigenic stimulation, their detection can be used as a tool for the early diagnosis of subclinical infection ([4]). Antibody measurement may also be utilized for the follow up of treatment, as an indirect but hopefully sensitive evaluation of the bacillary load.

Several antibody assays have been developed, some of them with the aim of detecting *Mycobacterium leprae*-specific antibodies for evolving a reliable diagnostic test. In the past, whole *M. leprae* or partially purified *M. leprae* extracts were used as antigens, with prior absorption of the sera to be tested on various mycobacterial preparations to eliminate crossreactivity with other species ([1, 9]). More recently, the discovery of an *M. leprae*-specific phenolic glycolipid (PGL-1), which could be extracted in large amounts from *M. leprae*-infected armadillo tissues, has opened up new perspectives in the field of leprosy serology ([2, 3, 5, 7, 19]). The other assays which used whole *M. leprae* or other mycobacteria and nonabsorbed sera did not attempt to detect species-specific antibodies for diagnostic purposes, but provided a global evaluation of the amount of antibody production in relation to bacillary load and disease activity ([12–14, 18]).

The aim of the present study was to compare both types of serological assays—*M. leprae*-specific and nonspecific—along the spectrum of the disease and, during the course of therapy, to measure by an ELISA assay IgM antibodies directed against the species-specific PGL-1, since this antigen has been shown to preferentially elicit IgM production ([6]), and IgM as well as IgG antibodies directed against whole *M. leprae.*

High titers of anti-PGL-1 IgM antibodies and of anti-whole *M. leprae* IgM and IgG antibodies were found in untreated lepromatous patients, with decreasing titers in treated patients. IgM antibodies, whether directed against PGL-1 or whole *M. leprae,* declined more rapidly than anti-whole *M. leprae* IgG antibodies (with wide variations from one patient to another).

Tuberculoid patients displayed a different antibody pattern with lower titers than lepromatous patients and a predominant production of anti-whole *M. leprae* IgG antibodies.

MATERIALS AND METHODS

Patients. Eighty-eight leprosy patients were followed in the Department of Dermatology, Hôpital Saint-Louis, in Paris. They were classified along the leprosy spectrum according to Ridley-Jopling's criteria ([17]) as polar lepromatous (LL), borderline lepromatous (BL), borderline tuberculoid (BT), and polar tuberculoid (TT). Some patients who were first seen by us when already treated for several years, and for whom no initial biopsy was available, were classified as lepromatous or tuberculoid on the basis of the clinical, immunological, bacteriological and histopathological data obtained at that time. In most patients, the bacterial index (BI) was evaluated as described by Ridley and Hilson ([16]) at the time of serum

[1] Received for publication on 26 September 1985; accepted for publication in revised form on 15 January 1986.

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collection on slit smears from the nasal mucosa, both ears, and one or two cutaneous lesions when present. The mean count from all sites studied was used for calculation of the BI.

Our study included a total of 49 lepromatous patients. Seven of them had never been treated when investigated for the first time. Three patients had suffered a clinical or bacteriological relapse either due to irregular treatment or acquired resistance to dapsone (DDS) and were thus also considered as untreated. Thirty-two lepromatous patients entered the study when already treated. Patients treated for less than four years received from the beginning a polychemotherapy consisting of various combinations of drugs, all regimens included rifampin: rifampin plus ethionamide plus clofazimine or DDS, or rifampin plus DDS and clofazimine. Twenty other patients had been treated for more than four years and received, at the time of study, either polychemotherapy according to one of the regimens described above, or DDS alone, or no treatment whatsoever. Except as otherwise noted, patients were not suffering from erythema nodosum leprosum (ENL) at the time of study.

Forty-two tuberculoid patients were also included in the present study. Eleven of them had never received treatment at the time of serum collection. Two patients suffering a clinical relapse with active lesions after stopping treatment were also considered as untreated. Twenty-nine patients entered the study after having already started treatment. Those treated for less than four years (nine patients) received polychemotherapy according to one of the schemes described above for lepromatous patients. Twenty patients treated for more than four years were receiving, at the time of study, either DDS or sulfadoxine, a long-term-acting sulfonamide, or no treatment.

Eighty-five healthy subjects living in Paris, either blood bank donors or staff members not exposed to *M. leprae,* served as controls.

Antigens. Phenolic glycolipid-1 (PGL-1) of *M. leprae,* extracted from *M. leprae*-infected armadillo liver ([7]), was a gift from the World Health Organization. Whole *M. leprae* suspensions were prepared by N. Rastogi (*M. leprae* bank, Pasteur Institut) from *M. leprae*-infected armadillo tissues (liver or lymph node) and were irradiated at 4.5 megarads with a ^{50}Co source before use.

Anti-PGL-1 antibody assay. The ELISA method described by Cho, *et al.* ([6]) was used with minor modifications. PGL-1 was dissolved in chloroform : methanol (2:1), emulsified in ammonium acetate/carbonate buffer (pH 8.2) by sonication for 10 sec, and adjusted to a final concentration of 2 μg/ml in the same buffer. Fifty μl aliquots of the PGL-1 suspension were distributed into the wells of flat-bottomed microtiter plates (Nunclon, Roskilde, Denmark) and incubated overnight at 37°C in a moist chamber. The plates were then washed with phosphate buffered saline (PBS), and incubated for 1 hr with PBS containing 5% w/v bovine serum albumin (PBS-BSA).

Fifty μl volumes of sera diluted 1:50 and 1:250 in PBS-BSA were introduced into the wells for 1 hr at 37°C. After washings, 50 μl of a 1 μg/ml solution of peroxidase-labeled goat anti-human μ chain antibodies (Biosys, Compiegne, France) was added to each well and further incubated overnight at 4°C in the dark. The plates were then washed and reacted with 100 μl of a solution of 0.4 mg/ml orthophenylethylenediamine dihydrochloride (OPD; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) in citrate phosphate buffer (0.15 M, pH 5.0) after which H_2O_2 solution was added to a final concentration of 0.013%. After 30 min at 37°C, the reaction was stopped by the addition of 50 μl of 2.5 N H_2SO_4. Optical densities (OD) were read at 492 nm. Each serum dilution was tested in duplicate on PGL-1-coated plates and on control plates incubated with carbonate buffer alone. Five reference sera (four positive lepromatous controls with various anti-PGL-1 activities and one normal serum used as a negative control) were included in each PGL-1-coated or uncoated plate. For each serum dilution, results were expressed as ΔOD = mean OD with PGL-1 − mean OD without PGL-1. Data obtained from plates in which the reference sera gave aberrant results were discarded.

Anti-whole *M. leprae* antibody assay. IgM and IgG antibody activities were concurrently measured for each serum. *M. leprae* were suspended in PBS, briefly sonicated (10 sec) to disrupt clumps, and diluted with PBS in order to obtain a suspension giving

THE TABLE. *Anti-*M. leprae *antibodies in untreated and long-term-treated leprosy patients.*

Antibody assay	Serum dilution	Subjects				
		Lepromatous patients		Tuberculoid patients		Healthy controls[a]
		Untreated	>10-year treated	Untreated	>10-year treated	
Anti-PGL-1 IgM antibodies	1:50	1031 ± 199[b] (8/10, 80%)[c]	568 ± 127 (6/13, 46%)	493 ± 120 (5/13, 38%)	209 ± 69 (1/11, 9%)	55 ± 26
	1:250	1560 ± 155 (10/10, 100%)	212 ± 50 (7/13, 54%)	178 ± 36 (6/13, 46%)	60 ± 23 (1/11, 9%)	33 ± 7
Anti-whole *M. leprae* IgM antibodies	1:250	1717 ± 145 (9/10, 90%)	946 ± 91 (2/13, 15%)	763 ± 48 (0/13, 0%)	608 ± 70 (0/11, 0%)	690 ± 29
	1:1000	1564 ± 141 (10/10, 100%)	508 ± 56 (2/13, 15%)	470 ± 34 (0/13, 0%)	387 ± 41 (0/11, 0%)	406 ± 18
Anti-whole *M. leprae* IgG antibodies	1:250	1420 ± 142 (9/10, 90%)	1122 ± 87 (10/13, 77%)	953 ± 127 (7/13, 54%)[d]	595 ± 110 (1/11, 9%)	428 ± 29
	1:1000	1560 ± 184 (10/10, 100%)	668 ± 102 (10/13, 77%)	555 ± 94 (6/13, 54%)[d]	243 ± 60 (1/11, 9%)	115 ± 17

[a] Seventy-one healthy controls were tested for anti-PGL-1 antibodies and 85 were tested for IgG and IgM anti-whole *M. leprae* antibodies.
[b] Mean ± S.E.M. ΔOD × 1000.
[c] In parentheses = number of positive sera/number of sera tested and % of sera positive.
[d] Eight out of 13 patients were found positive when both dilutions were considered.

an OD of 0.5 at 420 nm. These suspensions containing from 2×10^6 to 1×10^7 acid-fast bacilli/ml were used as the antigen in an ELISA assay similar to the radioimmunoassay described by Touw, *et al.* ([18]). Twenty-five μl volumes of the *M. leprae* suspension were distributed into round-bottomed microtiter plates (Imulon 2; Dynatech Laboratories, Alexandria, Virginia, U.S.A.) and allowed to dry overnight at 37°C. The bacilli were then fixed with glutaraldehyde (1% in PBS) for 15 min at room temperature. The plates were washed with PBS, and incubated with PBS-BSA for 1 hr at 37°C. Sera diluted at 1:250 and 1:1000 were added to the plates and kept for 1 hr at 37°C. The plates were washed and then incubated overnight at 4°C (in the dark) with peroxidase-labeled goat anti-human μ chain or γ chain antibodies (Biosys) diluted to 1 μg/ml in PBS-BSA. After rinsing, the plates were reacted with an OPD solution as described above, and the OD values were read at 492 nm. Each serum dilution was tested in duplicate on *M. leprae*-coated plates and on control plates with no antigen but similarly treated with glutaraldehyde. The same reference sera as those used for the anti-PGL-1 antibody measurements were tested on each plate as positive and negative controls. Results were expressed as ΔOD = mean OD with *M. leprae* − mean OD without *M. leprae.* Data obtained from the plates in which reference sera gave aberrant results were discarded.

Statistics. The Student *t* test was used to compare the means of the results obtained from the various groups of subjects. Regression lines and coefficients of correlation were calculated according to the least-square method. A serum was considered as "positive" in a given assay when the ΔOD exceeded by two standard deviations the mean of results obtained from normal controls in the same assay at the same dilution.

RESULTS

Antibody production along the leprosy spectrum: influence of long-term treatment (The Table). Only a minor binding activity to PGL-1 was found in the normal controls; the same group displayed a higher binding activity to whole *M. leprae,* the highest one being observed among IgM.

As compared to the normal controls, untreated lepromatous patients exhibited significantly higher antibody levels in all three assays. No significant decline of ΔOD was observed when the highest serum dilution was compared to the lowest (1:250 and 1:50,

respectively, for the anti-PGL-1 antibody assays; 1:1000 and 1:250, respectively, for both the IgM and IgG anti-whole *M. leprae* antibody assays). Moreover, several sera actually showed the highest activity with the highest dilution, which was due to the elevated spontaneous binding activity of the sera to the uncoated control wells when tested at the lowest dilution. The group of untreated lepromatous patients included 5 BL and 5 LL patients with no difference in the results between the two groups in any assay (data not shown). Lepromatous patients who had been treated for more than ten years displayed lower antibody activity in all three assays as compared to untreated cases ($p < 0.001$). However, 54% of these patients still exhibited a level of anti-PGL-1 activity which was significantly higher than normal controls, and as many as 77% of them were positive for IgG antibodies against whole *M. leprae.* A few patients (15%) maintained a detectable IgM antibody level against the whole bacillus.

Untreated tuberculoid patients (all BT) also displayed higher anti-PGL-1 antibody activity than the control group for both serum dilutions tested ($p < 0.001$), although the values were lower than those observed in untreated lepromatous patients. At variance with the lepromatous patients, the highest antibody activity was observed with the lowest serum dilution (most concentrated serum) in all but one patient. A total of 46% of the patients were positive in this assay.

Anti-whole *M. leprae* IgG antibodies could also be detected in tuberculoid patients. Most of them (11 of 13) showed the highest OD values with the 1:250 serum dilution; whereas 2 others paradoxically exhibited higher values with the highest serum dilution (1:1000), as did many untreated lepromatous patients. When the results from both dilutions were considered, 62% (8 out of 13) of the untreated tuberculoid patients were positive in this assay. Conversely, no IgM activity against whole *M. leprae* could be detected in any of these patients. Altogether, the antibody pattern in untreated tuberculoid patients was found to be very similar to that of long-term-treated lepromatous patients. Among the tuberculoid patients treated for more than ten years, only 1 of 11 still displayed detectable anti-PGL-1 antibodies; whereas another was positive for IgG anti-whole *M. leprae.*

Evolution of anti-PGL-1 and anti-whole *M. leprae* antibody titers in lepromatous patients during therapy. Lepromatous patients were subgrouped according to the time elapsed after a treatment regimen was instituted which caused a reduction of the bacterial load. Antibody titers were expressed as the ΔOD observed with the highest serum dilution tested. As shown in Figure 1, a significant drop of IgM anti-whole *M. leprae* antibodies could already be noted in the group of patients who were treated for 3 to 11 months ($p < 0.02$) as compared to untreated patients. Patients treated for more than two years did not show, as a group, any significant increase of antibody level in this assay as compared to the control group, although some individual sera were found to be positive. A significant decrease of anti-PGL-1 antibodies ($p < 0.001$) could also be observed, as compared to untreated patients from 12 months after onset of therapy, but without further decline.

In contrast to the antibody activities measured by both of these assays, IgG anti-*M. leprae* activity decreased very slowly after treatment had been started. Only those patients treated for more than two years showed a significant decrease in antibody level as compared to untreated patients.

Three patients were tested more than once, and detailed results from two are presented below. The third patient was studied at eight and 12 months after starting treatment, and during this time showed a small decrease of anti-PGL-1 antibody levels without significant change in anti-whole *M. leprae* antibody activities.

Longitudinal study of two lepromatous patients from the onset of therapy (Fig. 2). One patient (Dia. . ., BL) was followed for five months, and showed initially elevated antibody activities in all three assays. After three months of treatment with rifampin, ethionamide, and clofazimine, a reactional episode with neuritis and fever occurred which was not influenced by thalidomide but regressed under corticotherapy. On the basis of histopathological data, it was considered as a mild reversion (reversal reaction). At that time, a profound transient decrease of all three antibody activities was noted. By five months of therapy the patient

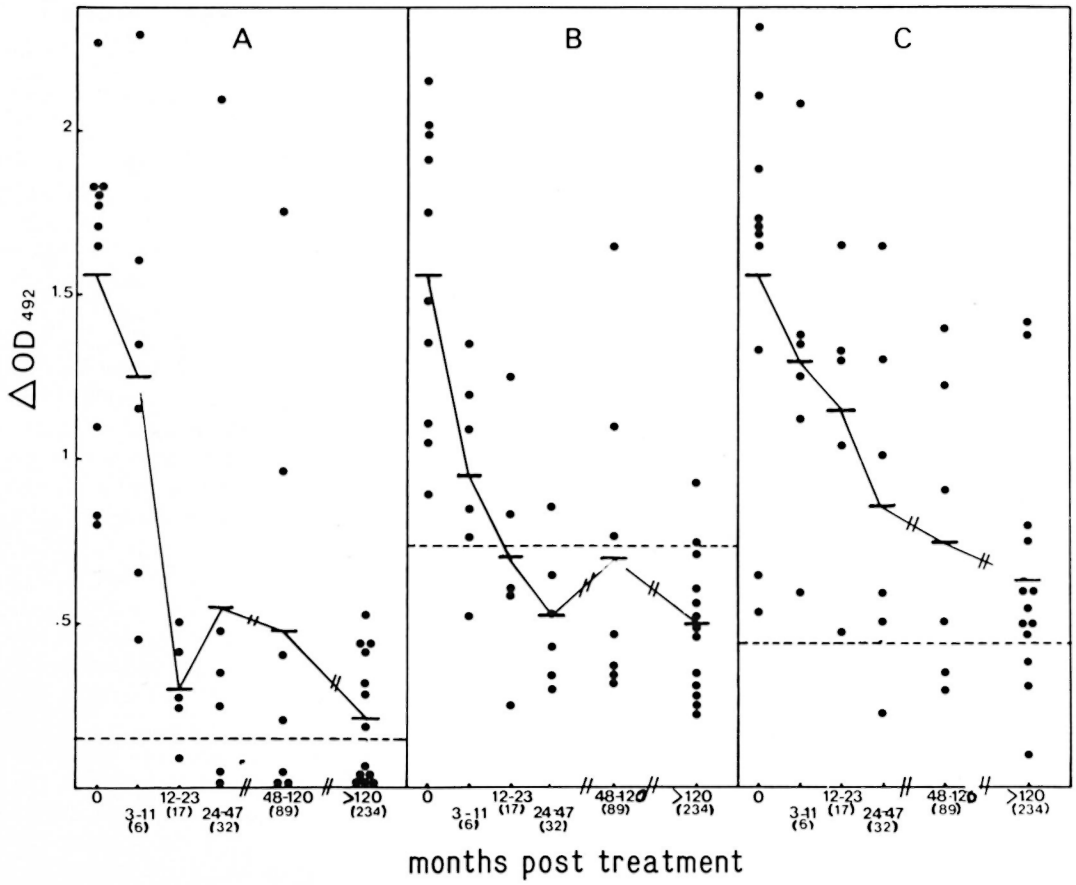

FIG. 1. Effect of treatment on anti-*M. leprae* activity in lepromatous patients. A = anti-PGL-1 IgM antibodies; B = anti-whole *M. leprae* IgM antibodies; C = anti-whole *M. leprae* IgG antibodies; horizontal bars represent the means of the subgroups of patients; (-----) = the 95% confidence upper limit of normal values.

was again clinically stable, with a BI which had dropped from 5.5 to 3.75, and he exhibited moderately reduced IgM antibody activities to PGL-1 and whole *M. leprae* when compared to pretreatment values. The level of IgG antibodies to whole *M. leprae* was similar to that recorded prior to treatment.

Another patient (Seu.., BL) was followed for 31 months after starting rifampin, ethionamide, and DDS therapy. Studied after 16 months, when suffering an ENL episode, a marked decrease of both IgG and IgM antibodies to whole *M. leprae* was noted, as well a small decrease of anti-PGL-1 antibodies as compared to pretreatment values. From 16 to 31 months of treatment, IgM antibody activity to whole *M. leprae* remained relatively low and stable. Fluctuations of IgG antibodies to whole *M. leprae* were also noted but without apparent relation to any clinical event. Interestingly, a transient decrease of anti-PGL-1 antibodies occurred again, and this was associated with an ENL reaction.

It must be noted that 5 long-term-treated patients (2 with lepromatous leprosy and 3 with tuberculoid leprosy), clinically and bacteriologically stable, were investigated twice at 2–4-week intervals. None of them showed variation of antibody activity by more than 25% in any assay.

Correlation of antibody titers with BI. Among lepromatous patients who had been treated for less than four years (Fig. 3), a weak but significant correlation was noted between the antibody level (as measured at the highest serum dilution) and the bacterial

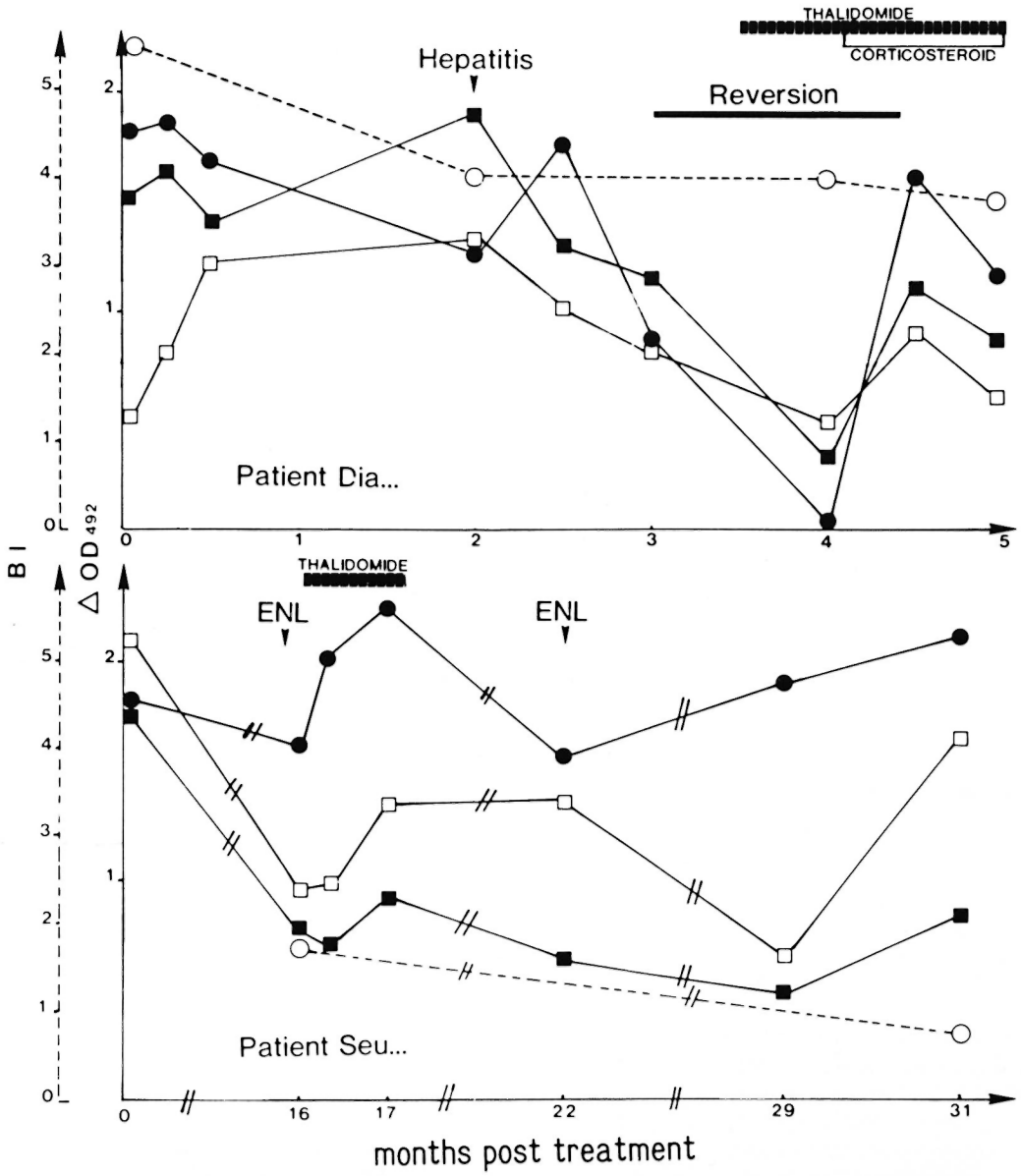

FIG. 2. Longitudinal follow up of anti-*M. leprae* antibody levels during therapy. Both patients were treated from time 0 with polychemotherapy (see text). Anti-PGL-1 IgM antibodies = ●-----●; anti-whole *M. leprae* IgM antibodies = ■-----■; anti-whole *M. leprae* IgG antibodies = □-----□; bacterial index (BI) = ○-----○.

load (as measured by the BI) for all three antibody assays (IgM anti-PGL-1: $r = 0.47$, $p < 0.05$; IgM anti-whole *M. leprae*: $r = 0.66$, $p < 0.01$; IgG anti-whole *M. leprae*: $r = 0.54$, $p < 0.02$). Positive correlations were found between antibody levels measured by these assays. However, when partial coefficients of correlation were calculated as a function of the BI, only IgM anti-PGL-1 and IgM anti-whole *M. leprae* antibody levels still appeared to be significantly (although weakly) correlated ($r = 0.465$, $p < 0.05$).

Among lepromatous patients treated for more than four years (Fig. 4), a correlation of BI with both IgM anti-PGL-1 ($r = 0.71$, $p < 0.01$) and IgM anti-whole *M. leprae* antibody level ($r = 0.67$, $p < 0.01$) was not-

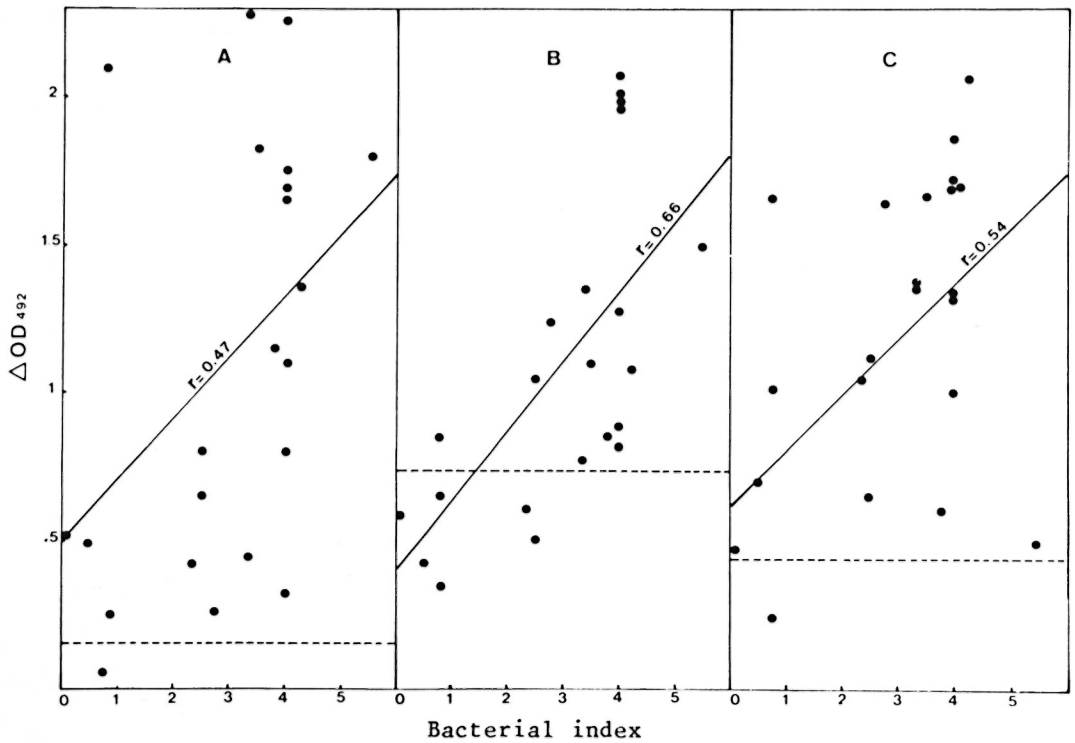

FIG. 3. Correlation of anti-*M. leprae* antibody titers to bacterial index (BI) in short-term-treated or untreated lepromatous patients. Sera were collected from untreated patients or patients treated for less than four years. Antibody titers are plotted against the BI. A = anti-PGL-1 IgM antibodies; B = anti-whole *M. leprae* IgM antibodies; C = anti-whole *M. leprae* IgG antibodies; (-----) = the 95% confidence upper limit of normal antibody titers; (——) = the best fitting regression lines; r = coefficient of correlation.

ed. Conversely, no correlation appeared in this group between BI and IgG anti-*M. leprae* antibody activity (r = 0.06).

Evolution of antibody levels in tuberculoid patients under therapy. Patients were subgrouped according to the time elapsed since onset of treatment, as shown in Figure 5. Results are expressed as ΔOD recorded with the lowest serum dilution, except for the few sera which gave higher values with the highest dilution, in which case the latter value was considered. Both untreated and short-term-treated tuberculoid patients showed a wide heterogeneity in serum antibody levels.

As in lepromatous patients, anti-PGL-1 antibody levels tended to decrease rapidly after treatment, since only 1 out of 6 patients treated for one to four years still exhibited a detectable antibody activity in this assay. One patient who had been tested before treatment showed a decline of anti-PGL-1 antibodies when tested eight months later. Three other patients who did not show elevated antibody activity when tested for the first time maintained normal values when studied for the second time.

IgG antibodies to whole *M. leprae* tended to decrease more gradually. Only those patients treated for more than four years had a significantly lower level of such antibodies when compared to untreated patients ($p < 0.05$). However, three patients studied twice during the first four years of treatment did exhibit a significant decline; whereas another patient showed a paradoxical increase of antibody level. No significant increase of IgM anti-whole *M. leprae* antibodies could be detected in any untreated or treated tuberculoid patients.

DISCUSSION

Antibodies of the IgM class directed against PGL-1, a species-specific, cell-wall associated antigen of *M. leprae* [2], and antibodies of both the IgM and the IgG classes

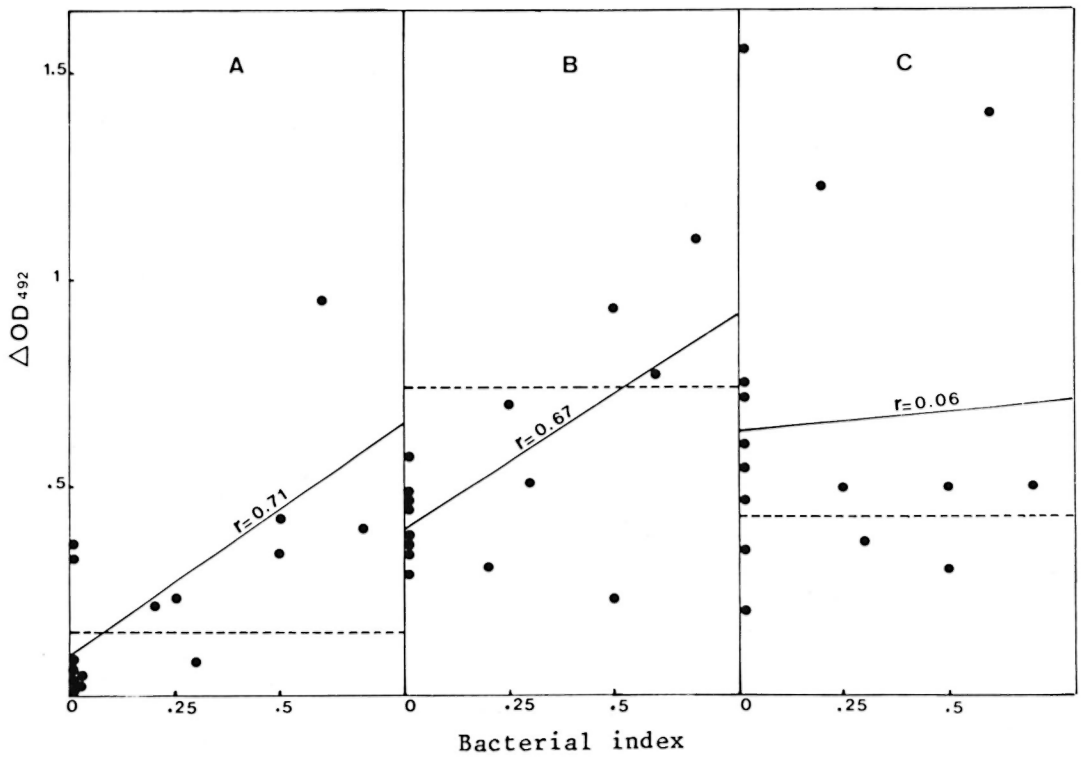

FIG. 4. Correlation of anti-*M. leprae* antibody titers to BI in long-term-treated lepromatous patients. Sera were collected from patients treated for more than four years. (See legend for Fig. 3.)

directed against whole *M. leprae* were measured by an ELISA method in sera from lepromatous and tuberculoid patients and normal subjects. Normal subjects who had no known exposure to *M. leprae* displayed very low IgM binding activity to PGL-1, in accordance with the previously reported exquisite specificity of this antigen with regard to *M. leprae* ([3, 6, 19]). Conversely, they showed significant antibody activity to whole *M. leprae,* especially of the IgM class, which might result from a previous exposure to crossreacting mycobacteria; indeed, BCG vaccination has been compulsory in France since 1950.

As compared to this group of normal subjects, untreated lepromatous patients exhibited a high level of anti-*M. leprae* antibodies in all three assays, which is again in agreement with several previous studies of anti-PGL-1 antibodies ([3, 6, 19]), anti-*M. leprae* sonicate antibodies ([9, 12]), and anti-whole *M. leprae* antibodies ([18]) in lepromatous leprosy. In the follow up of anti-*M. leprae* antibodies in treated lepromatous patients, we noted interesting variations in the pattern of decline of antibody levels according to the assay considered. A rapid fall of IgM antibodies directed either to PGL-1 or to whole *M. leprae* was observed within a year after onset of treatment, but noticeable individual variations were seen. On the other hand, IgG antibodies to whole *M. leprae* declined more slowly. Moreover, although antibody levels as measured by all three assays were found to correlate to the BI and to each other, the only significant correlation remaining when this effect of bacillary load was eliminated was a weak correlation between anti-PGL-1 and anti-whole *M. leprae* IgM antibody levels. These data suggest that, as expected, IgM antibodies against whole *M. leprae* include some IgM antibodies directed to PGL-1. In addition, there appear to be other antibodies directed against unrelated antigens, probably polysaccharides or lipids, which elicit the production of antibodies of the IgM class more than proteins ([15]). Conversely, anti-whole *M. leprae* antibodies of the IgG class would

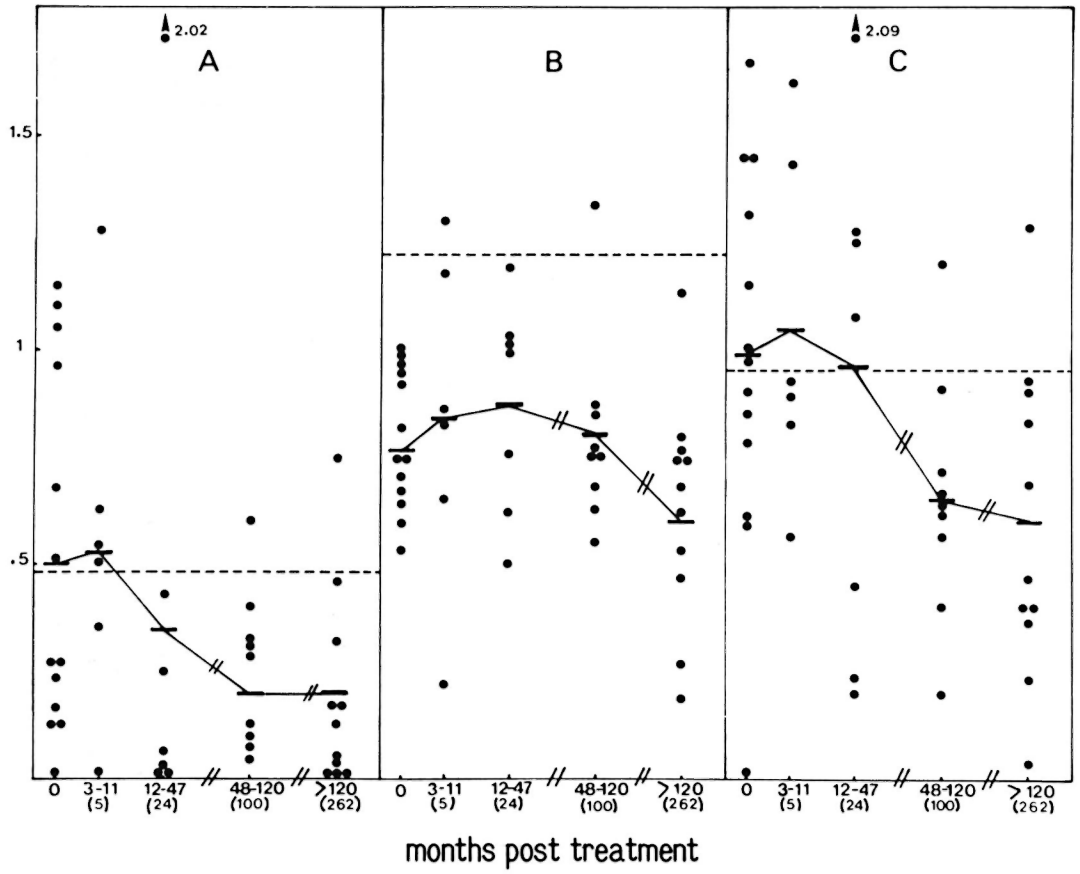

FIG. 5. Effect of treatment on anti-*M. leprae* antibody activity in tuberculoid patients. A = anti-PGL-1 IgM antibodies; B = anti-whole *M. leprae* IgM antibodies; C = anti-whole *M. leprae* IgG antibodies. (See legend for Fig. 1.)

recognize different types of antigens, including proteins.

Taken together, these data indicate that the follow up of anti-PGL-1 IgM antibody levels during the first years of therapy may be used as a rapid and sensitive tool for evaluating treatment efficacy, at least in nonreactional patients. It is interesting to note that our group of untreated patients actually included three relapsing patients who displayed antibody levels quite similar to those observed in recently diagnosed patients, suggesting that anti-PGL-1 antibody measurement may also help in the early detection of relapse.

A large percentage of lepromatous patients treated for more than four years still presented with significant antibody activity against PGL-1 or whole *M. leprae* (of the IgG class only in the latter case). In this group of patients, whose BI ranged from 0 to 1, anti-PGL-1 antibodies but not anti-whole *M. leprae* IgG antibodies were found to be closely correlated to the BI, suggesting that anti-PGL-1 antibodies probably represent a better indication of a persisting *M. leprae* infection than IgG antibodies against the whole bacillus. On the other hand, IgG anti-whole *M. leprae* antibodies could represent a reliable tool for retrospective detection of *M. leprae* infection in epidemiologic studies. Our data are in close agreement with those of Touw, *et al.* [18] who found a rapid decrease of IgM antibodies to whole *M. leprae* within a year, with persisting high titers of IgG antibodies, but contradict those of Melsom, *et al.* [12] who observed in ten-year-treated lepromatous patients a persisting antibody production of the IgM class but not of the IgG

class. It must be noted that Melsom, *et al.* used a soluble *M. leprae* sonicate as antigen; whereas, Touw, *et al.* ([18]) and ourselves used whole *M. leprae* suspensions, likely to contain a different proportion of cell-wall-associated versus cytoplasmic antigens.

Tuberculoid patients displayed an antibody pattern quite similar to that of long-term-treated lepromatous patients, that is, a significant production of IgM anti-PGL-1 antibodies and of IgG anti-whole *M. leprae* antibodies (but at a lower level and with a lower frequency than untreated lepromatous patients) without detectable IgM antibodies against whole *M. leprae*. The mechanism underlying individual variations of antibody production in tuberculoid patients—variations which have been previously emphasized by other workers ([18])—despite their apparent clinical and histopathological homogeneity is poorly understood. The presence of anti-PGL-1 antibodies in some patients may indicate the existence of a higher bacterial load than in negative patients. Touw, *et al.* ([18]) previously noticed a correlation of anti-whole *M. leprae* antibody titers with the number of active lesions in tuberculoid patients.

It is interesting to note that 4 out of 13 untreated BT patients were found positive for IgG anti-whole *M. leprae* antibodies and not for IgM anti-PGL-1 antibodies; whereas only 1 patient showed the reverse pattern. If some of this IgG antibody production is directed against species-specific antigens, as has been observed in *M. leprae*-immunized mice from which *M. leprae*-specific IgG secreting hybridomas were obtained ([8, 10, 11]), the use of such an antigen concurrently with PGL-1 would then significantly extend the efficacy of early serological detection of leprosy.

SUMMARY

Sera from 92 patients were tested by the ELISA method for the presence of IgM antibodies to phenolic glycolipid-1 (PGL-1) of *Mycobacterium leprae*, and of both IgM and IgG antibodies to the whole *M. leprae* bacillus. All untreated lepromatous patients exhibited high antibody levels in all three assays. A sharp decline of IgM antibodies to PGL-1 and whole *M. leprae* was observed during the first two years of therapy, while IgG antibodies to whole *M. leprae* showed a progressive decrease only over a number of years. Low titers of IgM antibodies to PGL-1 and IgG antibodies to whole *M. leprae* could be detected in about 50% and 75% of patients, respectively, after more than ten years of treatment, with only 15% showing persisting IgM antibodies to the whole bacillus.

Antibody levels as measured by the three assays used were correlated with the bacterial index in patients treated for less than four years. In patients treated longer than four years, only IgM antibodies, whether directed to PGL-1 or to whole *M. leprae*, remained correlated to the bacillary load. Tuberculoid patients exhibited a different antibody pattern, showing a lower frequency (and lower levels) of antibodies of PGL-1 and of IgG antibodies to whole *M. leprae* than lepromatous patients, and no detectable IgM antibodies to the whole bacillus. IgG antibodies to whole *M. leprae* were more frequently noted than antibodies to PGL-1, the latter declining more rapidly during therapy.

RESUMEN

Se adaptó el método de ELISA para investigar la presencia de anticuerpos contra el glicolípido fenólico 1 (GLF-1) del *Mycobacterium leprae* y la clase de anticuerpos (IgM o IgG) contra los bacilos íntegros en el suero de 92 pacientes con lepra. Todos los pacientes lepromatosos sin tratamiento tuvieron niveles elevados de anticuerpos en todos los ensayos. Mientras que los anticuerpos IgM contra el GLF-1 y contra el *M. leprae* íntegro decayeron bruscamente dentro de los 2 primeros años de tratamiento, los anticuerpos IgG contra el *M. leprae* sólo disminuyeron progresivamente a lo largo de varios años. Después de más de 10 años de tratamiento, el 50% de los pacientes tuvieron títulos bajos de anticuerpos IgM contra el GLF-1, el 75% de los pacientes tuvieron niveles bajos de anticuerpos IgG contra el *M. leprae* íntegro, y sólo el 15% de los mismos tuvieron títulos bajos de anticuerpos IgM contra el bacilo íntegro.

Los niveles de anticuerpos medidos por los 3 ensayos correlacionaron con el índice bacteriológico en los casos con menos de 4 años de tratamiento. En los pacientes con más de 4 años de tratamiento sólo correlacionaron con la carga bacilar, los anticuerpos IgM contra el GLF-1 o contra el *M. leprae* íntegro. A diferencia de los pacientes lepromatosos, los pacientes tuberculoides tuvieron una menor frecuencia y menores títulos de anticuerpos IgG contra el *M. leprae* y contra el GLF-1 y no tuvieron anticuerpos IgM contra

el bacilo íntegro. Los anticuerpos IgG contra el *M. leprae* íntegro fueron más frecuentes que los anticuerpos contra el GLF-1 y éstos últimos disminuyeron más rápidamente durante el tratamiento.

RÉSUMÉ

Des échantillons de sérum prélevés chez 92 malades ont été étudiés par la méthode ELISA en vue de mettre en évidence la présence d'anticorps IgM au pheno glycolipide-1 (PGL-1) de *Mycobacterium leprae*, ainsi que les anticorps IgM et IgG dirigés contre le bacille *M. leprae* complet. Tous les malades lépromateux non traités témoignaient de taux élevés d'anticorps pour les trois épreuves. Une diminution très prononcée des anticorps IgM contre PGL-1 et contre *M. leprae* complet a été observée au cours de deux premières années du traitement. Par contre, les anticorps à *M. leprae* complet montraient une diminution progressive qui s'étendait sur plusieurs années. Des titres peu élevés d'anticorps IgM contre PGL-1 et des anticorps IgG à *M. leprae* complet pouvaient être décelés chez environ 50% des malades pour les premiers et 75% chez les seconds après plus de dix ans de traitement. D'autre-part, 15% seulement des malades présentaient des anticorps IgM persistants contre le bacille entier.

Les taux d'anticorps, tels qu'ils sont mesurés par les trois épreuves utilisées, étaient en corrélation avec l'indice bactériologique des malades, lorsque que ceux-ci avaient été traités pendant moins de quatre années. Chez les malades traités pendant une période dépassant quatre ans, seuls les anticorps IgM, tant ceux dirigés contre PGL-1 que ceux dirigés contre *M. leprae* complet, étaient corrélés avec la charge bacillaire. Les malades tuberculoides ont révélé un profil d'anticorps différent, à savoir une fréquence plus faible, de même que des taux plus faibles, d'anticorps contre PGL-1 et d'anticorps IgG contre *M. leprae* complet, que ce n'était le cas pour les malades lépromateux. Aucun anticorps IgM n'a pu être décelé contre le bacille complet chez ces malades. On notait plus souvent la présence d'anticorps IgG contre le bacille complet, que d'anticorps contre PGL-1; ces derniers diminuaient plus rapidement au cours du traitement.

Acknowledgment. The excellent technical assistance of G. Dumas is gratefully acknowledged.

REFERENCES

1. Abe, M., Minagawa, F., Yoshino, Y., Ozawa, T., Saikawa, K. and Saito, T. Fluorescent leprosy antibody absorption (FLA-ABS) test for detecting sub-clinical infection with *M. leprae*. Int. J. Lepr. **48** (1980) 109–119.
2. Brennan, P. J. and Barrow, W. W. Evidence for species-specific lipid antigens in *M. leprae*. Int. J. Lepr. **48** (1980) 382–387.
3. Brett, S. J., Draper, P., Payne, S. N. and Rees, R. J. W. Serological activity of a characteristic phenolic glycolipid from *Mycobacterium leprae* in sera from patients with leprosy and tuberculosis. Clin. Exp. Immunol. **52** (1983) 271–279.
4. Buchanan, T. M., Young, D. B., Miller, R. A. and Khanolkar, S. R. Serodiagnosis of infection with *Mycobacterium leprae*. Int. J. Lepr. **51** (1984) 524–530.
5. Cho, S.-N., Fujiwara, T., Hunter, S. W., Rea, T. H., Gelber, R. H. and Brennan, P. J. Use of an artificial antigen containing the 3,6-di-O-methyl-β-D-glucopyranosyl epitope for the serodiagnosis of leprosy. J. Infect. Dis. **140** (1984) 311–322.
6. Cho, S.-N., Yanagihara, D. L., Hunter, S. W., Gelber, R. H. and Brennan, P. J. Serological specificity of phenolic glycolipid 1 from *M. leprae* and use in serodiagnosis of leprosy. Infect. Immun. **41** (1983) 1077–1083.
7. Draper, P., Payne, S. N., Dobson, G. and Minnikin, D. E. Isolation of a characteristic phthiocerol dimycoserate from *Mycobacterium leprae*. J. Gen. Microbiol. **129** (1983) 859–863.
8. Gillis, T. P. and Buchanan, T. M. Production and partial characterization of monoclonal antibodies to *Mycobacterium leprae*. Infect. Immun. **37** (1982) 172–178.
9. Harboe, M., Closs, O., Bjune, G., Kronvall, G. and Axelsen, N. H. *M. leprae*-specific antibodies detected by radio-immunoassay. Scand. J. Immunol. **7** (1978) 111–120.
10. Ivanyi, J., Sinha, S., Aston, R., Cussell, D., Keen, M. and Sen Gupta, U. Definition of species specific and cross-reactive antigenic determinants of *Mycobacterium leprae* using monoclonal antibodies. Clin. Exp. Immunol. **52** (1983) 528–536.
11. Kolk, A. H. J., Ho, M. L., Klatser, P. R., Eggelte, T. A., Kuijper, S., De Jonge, S. and Van Leeuwen, J. Production and characterization of monoclonal antibodies to *Mycobacterium tuberculosis, M. bovis* (BCG) and *M. leprae*. Clin. Exp. Immunol. **58** (1984) 511–521.
12. Melsom, R., Harboe, M., Myrvang, B., Godal, T. and Belehu, A. Immunoglobulin class-specific antibodies to *M. leprae* in leprosy patients, including the indeterminate group, and healthy contacts as a step in the development of methods for serodiagnosis of leprosy. Clin. Exp. Immunol. **47** (1982) 225–233.
13. Merklen, F. P. and Cottenot, F. Présence d'anticorps dans le sérum des lépreux. Bull. Soc. Pathol. Exot. Filiales **6** (1969) 982–987.
14. Miller, R. A., Harnisch, J. P. and Buchanan, T. M. Antibodies to mycobacterial arabinomannan in leprosy: correlation with reactional states and variation during treatment. Int. J. Lepr. **52** (1984) 133–139.
15. Perlmutter, R. M., Hansburg, D., Briles, D. E., Nicolotti, R. A. and Davie, J. M. Subclass restriction of murine anti-carbohydrate antibodies. J. Immunol. **121** (1978) 566–572.
16. Ridley, D. S. and Hilson, G. R. A logarithmic

index of bacilli in biopsies. Int. J. Lepr. **35** (1967) 184–193.

17. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity. A five-group system. Int. J. Lepr. **34** (1966) 255–273.
18. Touw, J., Lagendijk, E. M. I., Stoner, G. L. and Belehu, A. Humoral immunity in leprosy: immunoglobulin G and M antibody responses to *M. leprae* in relation to various disease patterns. Infect. Immun. **36** (1982) 885–892.
19. Young, D. B. and Buchanan, T. H. A serological test for leprosy with a glycolipid specific for *M. leprae.* Science **221** (1983) 1057–1059.