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Evaluation of four semi-synthetic *Mycobacterium leprae* antigens with sera from healthy populations in endemic and non-endemic areas

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Summary In order to determine the frequency of occurrence of antibodies to semisynthetic antigens of *Mycobacterium leprae* in clinically healthy nonpatient populations and to establish a 'baseline' for comparison with antibody frequencies in both patients with a history of leprosy and their contacts, ELISAs were conducted using representative sera from two areas: a leprosy endemic area, Cebu City, Philippines and a nonendemic area for leprosy Chicago, Illinois, USA. These sera were tested, by an indirect IgM ELISA, for the presence of antibodies reacting with four semisynthetic antigens based on the phenolic glycolipid I antigen of *M. leprae*: ND-O-BSA (natural disaccharide with octyl linkage to bovine serum albumin), NT-O-BSA (natural trisaccharide with octyl linkage to BSA), ND-P-BSA (natural disaccharide with phenolic ring linkage to BSA) and NT-P-BSA (natural trisaccharide with phenolic ring linkage to BSA). Using an OD reading $\geqslant 0{\cdot}16$ as positive, the antigen with the lowest background seroreactivity was ND-O-BSA, which reacted with 5/398 (1·3%) sera from Cebu, and 3/426 (0·7%) sera from Chicago. A total of 10 (2·5%) of 398 sera from the endemic area reacted with at least one antigen and 5 (1·3%) sera reacted with all four semisynthetic antigens. Of the 426 sera from Chicago, 12 (2·8%) were reactive with at least one antigen and 3 (0·7%) were reactive with all four semisynthetic antigens. Mean ELISA values for the 22 positive sera for each antigen ranged from 0·17 to 0·3 OD units, while the mean values for all sera in

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each area ranged from 0·01 to 0·04 OD units for all four antigens. Reactivity of 14 of the positive sera to some antigens, but not all four semisynthetic antigens, indicated that the carrier and linker arms might be associated with this background reactivity. Investigation of alternative linker arms and carriers is warranted. We conclude that nonspecific background reactivity to the semisynthetic antigens representing the PG-I molecule of *M. leprae* is 0·7–1·3%, based on a $\geqslant$0·16 OD cutoff value. From these data it was concluded that reactivity in individuals free of leprosy was low enough to warrant use of these antigens in a diagnostic setting, such as screening household contacts and highly endemic populations. When incidence and prevalence of leprosy are low, testing with these antigens would not be cost effective, unless applied to high risk individuals. Serological screening with these antigens might be useful in detecting and differentiating bacteriological relapse, type 1 or 2 reactions, early detection of leprosy, and monitoring treatment in endemic areas.

Introduction

The incubation period for leprosy has been estimated to vary between 2·9 and 5·3 years for tuberculoid leprosy and 9·3 and 11·6 years for lepromatous leprosy.[1] The disease may be transmitted to others during this long incubation period. Therefore, serological assays, which would indicate infection by detecting *Mycobacterium leprae* antibodies in asymptomatic individuals could facilitate the initiation of appropriate chemotherapy and assist control programmes by identifying potentially infectious individuals. Knowledge of background levels of antibody in the healthy endemic populations can also provide a baseline of reactivity to evaluate the prevalence of subclinical infection. Serological assays may be useful in patients with a history of leprosy, in evaluating the success of therapy, and in evaluating bacteriological relapse. Candidate antigens for such an assay include the semisynthetic neoglycoproteins, which mimic the sugar determinants of the phenolic glycolipid (PGL-I) of *M. leprae*.[2–7]

While these semisynthetic glycoprotein antigens have been studied in paients with disease, comparative survey results of reactivity among large numbers of healthy individuals in endemic and nonendemic areas have not been reported.[8–15] Information on the specificity and background levels of antibody in ELISA (enzyme-linked immunosorbent assay) with these antigens is needed to evaluate the serological prevalence of response to the PG-I synthetic analogues. This information must be derived from healthy populations in endemic areas. As suggested by Harboe, the controls used in antibody studies should be individuals with the same ethnic and socioeconomic background as the leprosy patients but who lack epidemiologic exposure to infectious cases.[16] However, establishing the specificity of a serological assay for a disease with such a long incubation period becomes problematic. It requires being able to follow a cohort of healthy individuals for many years after obtaining their sera to verify who does and who does not develop the disease. While the probability of becoming infected is negligible in a nonendemic area, there is a risk in an endemic area. Furthermore, healthy individuals in an endemic area without an epidemiological history of exposure may remain asymptomatic. Even if members of a cohort later developed disease, it would be unclear if they were infected at the time of the serum collection or if they became infected sometime after the sample was collected. Not being able to establish accurately when someone actually becomes infected and correlate this with the serological results complicates calculations of

test specificity. To adjust for this difficulty a nonendemic population with a near zero risk of developing disease was included in this study to assure a disease-free background level of antibody reactivity to these semisynthetic antigens.

The frequencies of antibody reactivity to 4 semisynthetic antigens of *M. leprae* in the endemic and the nonendemic populations are presented in this paper from data generated by an IgM specific indirect ELISA. The 4 semisynthetic antigens are compared for degree of reactivity in the study populations. The nonendemic population sera were obtained from health care personnel and obstetric patients from Chicago, Illinois, USA. The seroreactivity rate in this group could then be compared to the seroreactivity rate among healthy individuals in an endemic population. The endemic population was selected from healthy individuals residing in Cebu City, Philippines, who attended the Cebu Skin Clinic for conditions other than leprosy and who did not have a leprosy case in their household.

Materials and methods

STUDY POPULATIONS

The Chicago population sample consisted of 426 healthy young adults (predominantly nursing students, medical students and obstetrical patients). None of these subjects had a history of residence in a leprosy endemic area or contact with a patients with active leprosy. In the Chicago population, 82% of the subjects were females and 18% were males. The Chicago females ($n = 351$) were between 11 and 81 years old, with a median age of 23 years. The Chicago males ($n = 75$) were between 17 and 58 years old, with a median age of 28 years (Table 1).

In the Cebu population of 398 persons, the sex distribution was even, with 199 males and 199 females. These individuals were selected from those people who came into the Cebu Skin Clinic for diseases other than leprosy, mostly for ordinary skin diseases such as eczema, fungal infections, bacterial and viral infections, acne vulgaris, and numerous other skin diseases. Those who were selected for the study did not have a leprosy case in their household and had no clinical evidence of leprosy at the time the blood was

Table 1. Age and sex distribution of the 2 study populations

Age category (Years)	Leprosy endemic (Cebu)		Leprosy nonendemic (Chicago)	
	Male	Female	Male	Female
11–20	45	35	1	110
21–30	70	66	48	186
31–40	30	44	21	48
41–50	20	22	4	5
51–60	18	19	1	0
61–70	10	11	0	1
71–80	6	2	0	0
81+	0	0	0	1
Total	199	199	75	351

collected. The females were between 13 and 77 years old, with a median age of 30 years. The males were between 13 and 78 years old, with a median age of 28 years (Table 1).

Antigens

Immulon II 'u' bottom plates (Dynatech Laboratories, Alexandria, VA 22314, USA) were coated with antigen in a volatile buffer as previously described.[17] The antigens, based on the phenolic glycolipid-I antigen of *M. leprae*, were: ND-O-BSA [natural disaccharide with ocytl linkage to bovine serum albumin (BSA)], NT-O-BSA (natural trisaccharide with octyl linkage to BSA), ND-P-BSA (natural disaccharide with a phenolic ring linkage to BSA) and NT-P-BSA (natural trisaccharide with phenolic ring linkage to BSA).[5–7] Microtitre plates were coated by applying 50 microlitres of a given antigen at a concentration of 0·2 micrograms/ml (based on the weight of the sugars). Unmodified BSA (Cat. No. A-7030, Sigma Chemical Co., St Louis, MO 63178, USA) was used as a control antigen and coated at a concentration of 0·32 μg/ml. The octyl linked antigens were provided under NIH contract (NO1 AI-52582) by Dr P. Brennan, Colorado State University, USA. The phenol-linked sugars were provided by Dr T. Fujiwara of Nara University with funding provided by the Western Regional Office of the Pacific area of the World Health Organization.

SERA

The sera were separated from blood collected by venipuncture and aliquots stored at $-70°$C until ready for use.

ELISA

The assay was performed as previously described.[18] Plates coated with the specified antigen were first blocked with 75 microlitres of 10% skimmed milk in phosphate-buffered saline with 0·1% Tween-20 (PBS), pH 7·4, and incubated for 1 hour at 37°C. The sera were diluted 1:500 in 10% skimmed milk in PBS, pH 7·4. Control sera (positive: lepromatous sera; negative: normal sera) were diluted similarly. Each plate included the same control sera: a high positive control (OD of $1{\cdot}30 \pm 0{\cdot}08$), a low positive control (OD of $0{\cdot}3 \pm 0{\cdot}04$) and 4 negative sera (1, a pooled sample and 3 from individuals with OD values less than 0·10).

Peroxidase conjugated goat anti-human IgM (μ-chain specific, Cappel Laboratories) was diluted 1:6000 in blocking buffer and incubated in the wells for 45 min at 37°C. Any study serum sample yielding an optical density of 0·10 or higher was retested twice in duplicate and the values of the 4 OD values were averaged. The conjugate background values were subtracted from all sera values prior to recording.

Results

COMPARATIVE ENDEMIC AND NON-ENDEMIC IgM REACTIVITIES BY SEX

The mean OD values ranged from $0{\cdot}02 \pm 0{\cdot}03$ to $0{\cdot}04 \pm 0{\cdot}05$ and the sex of the study subjects made no difference in either the endemic or nonendemic populations. We detected no significant differences in reactivity to the various antigens when the subjects

were stratified by age and sex. The NT-O-BSA antigen had slightly higher background (0·01 to 0·02 OD units greater) than the other antigens in both populations. Also females in the 21–30 and 31–40-year-old age groups were slightly more reactive than males and other age groups with OD values of 0·01–0·02 units higher.

Comparisons by population and sex were also made for those samples which were positive to at least one of the antigens. Positive sera were those with reactivity greater than 0·15 OD units. The means ± SD for the positive tests were: 0·21 ± 0·02 for ND-O-BSA, 0·23 ± 0·03 for NT-O-BSA, 0·17 ± 0·01 for ND-P-BSA, and 0·2 ± 0·01 for NT-P-BSA. For the endemic population (Table 2), 4 males had sera which were reactive to at least 1 of the antigens. For the females, 6 of the sera were reactive to at least 1 of the antigens, and those means ± SD for positive reactions were: 0·24 ± 0·08 for ND-O-BSA, 0·23 ± 0·07 for NT-O-BSA, 0·23 ± 0·09 for ND-P-BSA, and 0·23 ± 0·04 for NT-P-BSA.

For the nonendemic population (Table 3), only 1 male had a serum sample that was reactive. It was reactive to NT-P-BSA at an OD value of 0·16. A total of 6 females were reactive to at least 1 of the antigens. The means ± SD of positive values for these samples were: 0·27 ± 0·13 for ND-O-BSA, 0·3 ± 0·14 for NT-O-BSA, 0·23 ± 0·05 for ND-P-BSA, and 0·25 ± 0·06 for NT-P-BSA. These values are similar to the values of the endemic positive reactive tests. The mean positive OD values for ND-O-BSA (0·27) and NT-O-BSA (0·3) appear to be higher than those to the other antigens. However, this is due to 1 sample which had high OD values of 0·46 and 0·55, respectively.

A total of 22 sera from the endemic and non-endemic populations, which were positive with at least 1 or more antigens, were retested with the unmodified BSA carrier

Table 2. ELISA IgM optical density values* for sera reactive to semisynthetic *M. leprae* antigens from an endemic area (Cebu, Philippines)

Serum No. (Sex/Age)	BSA only	Semisynthetic antigens				Number positive
		ND-O-BSA	NT-O-BSA	ND-P-BSA	NT-P-BSA	
146 (F/22)	0·04	**0·20**	**0·19**	**0·17**	**0·19**	4
190 (F/31)	0·03	**0·19**	**0·20**	**0·21**	**0·21**	4
300 (M/72)	0·00	**0·22**	**0·22**	**0·16**	**0·19**	4
519 (M/21)	0·03	**0·19**	**0·26**	**0·18**	**0·20**	4
815 (F/62)	0·04	**0·34**	**0·35**	**0·35**	**0·27**	4
693 (F/50)	0·04	0·14	**0·18**	**0·17**	0·11	2
105 (F/37)	0·03	0·08	**0·30**	0·08	**0·25**	2
297 (M/61)	0·03	0·13	**0·20**	0·08	0·07	1
341 (F/35)	0·04	0·09	**0·18**	0·04	0·06	1
514 (M/37)	0·04	0·12	**0·22**	0·12	0·10	1
No. positive (%†)		5 (1·3)¶	10 (2·5)	6 (1·5)	6 (1·5)	27
Strong positive‡		1 (0·25)	2 (0·5)	1 (0·25)	0 (0)	
Weak positive§		4 (1·0)	8 (2·0)	5 (1·25)	6 (1·5)	

* Average values resulting from 4 assays; positive values (OD ≥ 0·16) in **bold** type. Control OD values were: high positive control = 1·30 ± 0·08, low positive = 0·3 ± 0·04 and negative sera = 0·04 ± 0·04.

† % = Number positive/398 tested × 100%.

‡ Strong positive = $OD_{492} \geqslant 0{\cdot}30$.

§ Weak positive = $0{\cdot}16 \leqslant OD_{492} \leqslant 0{\cdot}29$.

¶ Numbers in parentheses are percentages.

(Tables 2 and 3). None of the sera was found to be positive to BSA. The mean ± SD of the OD values for these sera was 0·03 ± 0·02.

IgM REACTIVITY IN AN ENDEMIC AREA

Table 2 presents ELISA values for all the Cebu sera that were reactive to at least 1 antigen according to the antigen(s) to which the sera were reactive. While serum no. 815 gave the highest reading, other sera tested did not yield strongly positive results, i.e. OD values ⩾0·30. Overall, 10 of the 398 sera (2·5%) were reactive to at least 1 of the 4 antigens, and all ten were reactive with NT-O-BSA; 5 sera (1·3%) had OD values of at least 0·17 to all 4 antigens and 2 sera (0·5%) were reactive to 2 antigens and 3 sera (0·7%) were reactive to only 1 antigen. In comparing only the samples with positive OD values, the average OD values were not significantly different between the antigens.

Table 2 also summarizes the positive serological reactivity of 398 people from Cebu City to each of the 4 semisynthetic antigens. A sample was considered to be a high positive if it yielded an OD value of ⩾0·30, was low positive if it gave an OD of 0·16–0·29, and was negative if the OD was ⩽0·15. The fewest number of sera (5/398) reacted to ND-O-BSA, the largest number of sera reacted to NT-O-BSA (10/398), and 6/398 sera reacted to both ND-P-BSA and NT-P-BSA.

Table 3. ELISA IgM optical density values* for sera reactive to semisynthetic *M. leprae* antigens from a non-endemic area (Chicago, USA)

Serum No. (Sex/Age)	BSA only	Semisynthetic antigens				Number positive
		ND-O-BSA	NT-O-BSA	ND-P-BSA	NT-P-BSA	
R10109 (F/25)	0·02	**0·21**	**0·22**	**0·23**	**0·20**	4
R10225 (F/18)	0·01	**0·46**	**0·55**	**0·30**	**0·31**	4
R10251 (F/17)	0·02	**0·23**	**0·30**	**0·21**	**0·18**	4
R9923 (F/19)	0·05	0·03	**0·21**	0·02	**0·20**	2
R9916 (F/37	0·01	0·01	0·01	**0·27**	**0·28**	2
R10190 (F/22)	0·02	0·13	0·14	**0·17**	0·09	1
R10121 (F/22)	0·07	**0·16**	0·14	0·15	0·04	1
R9996 (F/21)	0·03	0·00	**0·33**	0·05	0·04	1
R9984 (F/32)	0·03	0·02	0·00	**0·20**	0·06	1
R9800 (F/20)	0·00	0·00	0·01	0·00	**0·32**	1
No. 88 (F/33)	0·01	0·13	**0·16**	0·04	0·01	1
No. 37 (M/39)	0·02	0·02	0·06	0·00	**0·16**	1
No. positive (%†)		4 (0·9)¶	6 (1·4)	6 (1·4)	7 (1·6)	23
Strong positive‡		1 (0·2)	3 (0·7)	1 (0·2)	2 (0·5)	
Weak positive§		3 (0·7)	3 (0·7)	5 (1·2)	5 (1·2)	

* Average values resulting from four assays; positive values (OD ⩾0·16) in **bold** type. Control OD values were: high positive control = 1·30 ± 0·08, low positive = 0·3 ± 0·04 and negative sera = 0·04 ± 0·04.

† % = Number positive/398 tested × 100%.

‡ Strong positive = OD_{492} ⩾0·30.

§ Weak positive = 0·16 ⩽ OD_{492} ⩽0·29

¶ Numbers in parentheses are percentages.

IgM REACTIVITY IN A NON-ENDEMIC AREA

Of the 426 sera tested from Chicago, 12 (2·8%) were reactive to at least 1 antigen (Table 3), 3 of the samples (0·7%) were reactive to all 4 antigens, 2 sera were (0·5%) reactive to 2 antigens, and 7 sera (1·6%) were each reactive to only 1 of the antigens. Only serum R9923 was reactive with the 2 trisaccharide antigens but not with the 2 disaccharide antigens. As with the Cebu sera, if only the positive OD values are compared, the average OD values were not significantly different between the antigens.

The positive serological responses of 426 people from Chicago to the 4 semi-synthetic antigens are also summarized in Table 3. As with the Cebu data, ND-O-BSA produced the lowest frequency of reactive sera (4/426) compared to NT-O-BSA (6/426), ND-P-BSA (6/426), and NT-P-BSA (7/426). The seropositivity rates of the Chicago sera were not significantly different from those from Cebu.

Discussion

We have studied the seroprevalence in IgM ELISAs of 4 semisynthetic *M. leprae* antigens in 2 populations, 1 endemic and 1 nonendemic for leprosy. This study allowed us to evaluate and compare the specificity of these 4 semisynthetic antigens as markers for subclinical infection.

The overall rates of sera reactive to at least 1 of the antigens were comparable between the endemic population (2·5%) and the nonendemic population (2·8%). More than half (7/12) of the nonendemic sera were reactive to only 1 of the antigens. For sera which reacted to all 4 antigens, the positive rate in the endemic area was 1·3% (5/398). Although this was almost double the rate of 0·7% (3/426) in the nonendemic area, these rates are not statistically significant.

The possible reaction of the sera with BSA has been questioned, but we found that when similar assay conditions were employed using BSA as the antigen, all the sera were nonreactive (Tables 2 and 3). The contributions of the 3rd sugar, the phenol linker arms, octyl linker arms and BSA were evaluated. The dominant sugars were the terminal and penultimate sugars and 8 of 832 sera reacted with all 4 antigens; 2 sera out of 836 individuals reacted only to the trisaccharide antigens (NT-O-BSA and NT-P-BSA): these were serum no. 105 from the endemic sera and serum R9923 from the nonendemic population; 1 serum, R9916, from the nonendemic group reacted only with the antigens containing the phenol linker arm. There was no indication that the BSA carrier was recognized by itself; 4 of the endemic sera and 7 of the nonendemic sera reacted with single antigens. Thus, 12 of the 22 reactive sera from 836 tested were responding to structures beyond the sugar epitopes. This may be due to the recognition of unique conformational structures formed between the BSA, linker arm and the sugar determinants. The antigen to which the most sera reacted was NT-O-BSA (16/22) and the least reactive was ND-O-BSA (9/22). From these data we conclude that improvements could be made with this family of antigens. Further development of other linker arms and carriers could reduce the reactivity in healthy individuals to the semisynthetic antigens representing the PG-I antigen.

A limitation of the study was that, since it was designed as a cross-sectional study, we did not follow-up serologically positive healthy individuals. In our previous studies of

leprosy patients we were able to follow up individual cases since the patients were being treated medically and followed in a clinic. It was not possible to follow up individuals in either of the present populations. This was because the participants in the endemic area were not under medical care, while the population from the nonendemic area was transient. These problems limited our ability to follow up and observe the serological conversion and reversion in healthy populations. However, the nonendemic group did provide baseline data in a population which allowed us to estimate the specificity of the various serological assays.

In one of our previous studies, we showed that the correlations between NT-O-BSA and ND-O-BSA ranged from $r = 0{\cdot}963$ to $0{\cdot}998$ ($p < 0{\cdot}001$) for 31 lepromatous patients in an endemic area, Cebu, PI.[14] In another study, we found that the reactivity rates of the patient sera from 35 lepromatous patients in an endemic area (Cebu, PI) to ND-O-BSA, and NT-O-BSA were highly correlated ($r = 0{\cdot}866$, $p < 0{\cdot}001$).[15] However, the seroprevalence to ND-P-BSA with patient sera was found to be lower than the other antigens.[19] Our findings in this and previous work with sera from patients with clinical leprosy have led us to concentrate on the use of ND-O-BSA antigen, since it provides better specificity and sensitivity than the NT-O-BSA and NT-P-BSA antigens. The NT-P-BSA antigen is similar in reactivity to ND-O-BSA. Of the 4 semi-synthetic antigens, ND-O-BSA appears to be the antigen of choice for the detection of subclinical disease or asymptomatic infections in this endemic population because of its lower background reactivity, i.e. greater specificity.

This report did not evaluate the D-BSA antigen described by Gigg *et al.*[3] However, the ND-O-BSA antigen and D-BSA antigens were compared in a report by Engers *et al.*[20] They found that the 2 antigens were similar in reactivity with 20 lepromatous sera and 7 control sera.

No significant differences could be found when the subjects were stratified by age and sex. This finding is not in agreement with those reported by Fine *et al.*[21] We found a slight increase, though not significant, in ELISA values for females compared to males in the 21–30 and 31–40-year-old age groups. The difference in findings with others may be explained by the dilution of sera in our test to 1:500, which could result in lowering of natural levels of IgM in the test sample. This seems reasonable since the dilutions from eluted blood imprenated paper in the report by Fine *et al.* were equivalent to 1:20 dilution of serum.[21] In another report, Izumi *et al.*, describing a particle agglutination test using the NT-P-BSA as antigen, reported a higher than expected rate of reactivity among normal pregnant women.[22] When the sera from the pregnant women was diluted 1:32, 12/120 were found to be positive. This reactivity could be eliminated by continuing the dilution of sera to 1:64.

In summary, this study has allowed us to estimate the specificity of 4 semisynthetic glycoconjugate *M. leprae* antigens in an endemic and a nonendemic population. Our data suggest that the specificity of these antigens is quite high, i.e. 98·7% in the leprosy endemic area. It is possible that the difference between the rate of false-positive tests (0·94%) in a nonendemic area and the rate of positive tests in seropositive healthy individuals in the endemic area reflects subclinical infection. In terms of practical application, we concluded that seropositivity in individuals free of leprosy was low enough to warrant use of these antigens in diagnostic settings, and in screening household and other contacts for subclinical leprosy infections. The specificity of the antigens may be improved by developing new carriers and/or linker arms for the synthetic sugar epitopes. Where the

risk, or incidence and prevalence of leprosy is low, it would not be cost effective to use these antigens in screening general populations, since the rate of false positives would greatly exceed the numbers of new cases detected.

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References

[1] National Communicable Disease Center, U.S. Department of Health, Education and Welfare/Public Health Service 1970 Leprosy Surveillance. January 1970, Report No. 1.

[2] Fujiwara T, Hunter SW, Cho SN, Aspinall GO, Brennan PJ. Chemical synthesis and serology of disaccharides and trisaccharides of phenolic glycolipid antigens from the leprosy bacillus and preparation of a disaccharide protein conjugate for serodiagnosis of leprosy. *Infect Immun*, 1984; **43:** 245–252.

[3] Gigg J, Gigg R, Payne S, Conant R. Synthesis of propyl4-*O*-(3,6-di-*O*-methyl-beta-D-glucopyranosyl)-2,3-di-*O*-methyl-alpha-D-rhamnopyranoside. *Carbohydr Res*, 1985; **141:** 91–97.

[4] Chatterjee D, Douglas JT, Cho SN, Rea TH, Gelber RH, Aspinall GO, Brennan PJ. Synthesis of neoglycoconjugates containing the 3,6-di-*O*-methyl-beta-D-glucopyranosyl epitope and use in serodiagnosis of leprosy. *Glycoconjugate J*, 1985; **2:** 187–208.

[5] Fujiwara T, Hunter SW, Brennan PJ. Chemical synthesis of disaccharides of the specific phenolic glycolipid antigens from *Mycobacterium leprae* and of related sugars. *Carbohydr Res*, 1986; **148:** 287–298.

[6] Fujiwara T, Aspinall GO, Hunter SW, Brennan PJ. Chemical synthesis of the trisaccharide unit of the species-specific phenolic glycolipid of *Mycobacterium leprae. Carbohydr Res*, 1987; **163:** 41–52.

[7] Chatterjee D, Cho SN, Stewart C, Douglas JT, Fujiwara T, Brennan PJ. Synthesis and immunoreactivity of neoglycoproteins containing the trisaccharide unit of the phenolic glycolipid I of *Mycobacterium leprae. Carbohydr Res*, 1988; **183:** 241–260.

[8] Anonymous. Serological tests for leprosy. (Editorial) *Lancet*, 1986; **1:** 533–535.

[9] Cho SN, Fujiwara T, Hunter SW, Rhea TH, Gelber RH, Brennan, PJ. Use of an artificial antigen containing the 3,6 di-*O*-methyl-D-glucopyranosyl epitope for the serodiagnosis of leprosy. *J Infect Dis*, 1984; **150:** 311–322.

[10] Brett SJ, Payne SN, Gigg J, Burgess P, Gigg R. Use of synthetic and immunodominant epitope of phenolic glycolipid I in serology of leprosy. *Clin Exp Immunol*, 1986; **64:** 476–483.

[11] Douglas JT, Cellona RV, Abalos RM, Madarang MG, Fajardo TT. The serological reactivity and early detection of leprosy among contacts of lepromatous patients in Cebu, Philippines. *Int J Lepr*, 1987; **55:** 718–721.

[12] Chanteau S, Cartel J-L, Roux R, Plicart R, Bach M-A. Comparison of synthetic antigens for detecting antibodies to phenolic glycolipid I in patients with leprosy and their household contacts. *J Infect Dis*, 1988; **157:** 770–776.

[13] Douglas JT, Steven LM, Fajardo TT, Cellona RV, Abalos RM, Steenbergen GJ. The effects of chemotherapy on antibody levels in lepromatous patients. *Lepr Rev*, 1988; **59:** 127–135.

[14] Douglas JT, Hirsch DS, Fajardo TT, Cellona RV, Abalos RM, Dela Cruz EC, Madarang MG, De Wit MYL, Klatser PR. Evaluation of *M. leprae* antigens in the serological monitoring of a clofazamine based chemotherapeutic study of dapsone resistant lepromatous leprosy patients in Cebu, Philippines. *Lepr Rev*, 1989; **60:** 8–19.

[15] Klatser PR, De Wit MLY, Fajardo TT, Cellona RV, Abalos, R.M, Dela Cruz EC, Madarang MG, Hirsch DS, Douglas JT. Evaluation of *M. leprae* antigens in the monitoring of a dapsone based chemotherapy of previously untreated lepromatous patients in Cebu, Phillippines. *Lepr Rev*, 1989; **60:** 178–186.

[16] Harboe M. The immunology of leprosy. *In: Leprosy*, R.C. Hastings, ed., Churchill Livingstone, Edinburgh, London, Melbourne and New York, 1985; 53–87.

[17] Douglas JT, Naka SO, Lee JW. Development of an ELISA for detection of antibody in leprosy. *Int J Lepr*, 1983; **52:** 19–25.

[18] Douglas JT, Wu QX, Agustin GP, Madarang MG. Evaluation of inexpensive blocking agents for ELISA in the detection of leprosy. *Lepr Rev* 1988; **59:** 37–43.

[19] WHO Report: Meeting of the Working Group on Rapid Diagnostic Test for Leprosy. World Health Organization, Regional Office for the Western Pacific, Manila, Philippines 7–8 July 1987 ((WP)CHD/ICP/LEP/001-E).

[20] Sanchez GA, Malik A, Tougne C, Lambert PH, Engers HD. Simplification and standardization of serodiagnostic tests for leprosy based on phenolic glycolipid-I (PG-I) antigen. *Lepr Rev*, 1986: **57,** Suppl 2, 83–93.

[21] Fine PEM, Ponnighaus JM, Burgess P, Clarkson JA, Draper CC. Seroepidemiological studies of leprosy in Northern Malawi based on an enzyme-linked immunosorbent assay using synthetic glycoconjugate antigen. *Int J Lepr*, 1988; **56:** 243–254.

[22] Izumi S, Fujiwara T, Ikeda M, Nishimura Y, Sugiyama K, Kawatsu K. Novel gelatin particle agglutination test for serodiagnosis of leprosy in the field. *J Clin Microbiol*, 1990; **28:** 525–529.

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Évaluation de quatre antigènes semi-synthétiques de *Mycobacterium leprae* avec les serum de populations saines dans des régions d'endémie et de non-endémie

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Résumé Notre but était de déterminer la fréquence de l'apparition d'anticorps aux antigènes semi-synthétiques de *Mycobacterium leprae* dans des populations de non-malades en bonne santé clinique et d'établir une base pour la comparaison de la fréquence des anticorps chez les patients avec une histoire de lèpre d'une part, et leurs contacts d'autre part. Nous avons donc exécuté des tests ELISA sur des serum représentant deux régions: une région où la lèpre est endémique, Cebu City, Philippines et une régon où la lèpre n'est pas endémique, Chicago, Illinois, USA. Ces serum ont été testés, par indirect IgM ELISA, pour rechercher la présence d'anticorps réagissant avec quatre antigènes semi-synthétiques basés sur l'antigène phénolique glycolipidique I de *M. leprae*: ND-O-BSA (disaccharide naturel lié par un octyl à la serum albumine de boeuf BSA), NT-O-BSA (trisaccharide naturel lié par un octyl à BSA), ND-P-BSA (disaccharide naturel lié par un noyau phénolique à BSA). En prenant 0,16 comme lecture OD positive, l'antigène avec la séroréactivité de fond la plus basse était ND-O-BSA, qui réagissait avec 5/398 (1,3%) serum provenant de Cebu, et 3/426 (0,7%) serum provenant de Chicago. Au total 10 des 398 serum (2,5%) provenant de la région d'endémie ont réagi avec au moins un antigène et 5 serum (1,3%) ont réagi avec tous les quatre antigènes semi-synthétiques. Des 426 serum provenant de Chicago, 12 (2,8%) ont réagi avec au moins un antigène et 3 (0,7%) avec tous les quatre antigènes semi-synthétiques. Les valeurs moyennes de l'ELISA pour les 22 serum positifs pour chaque antigène ont varié entre 0,17 et 0,3 unités OD, tandis les valeurs moyennes pour tous les serum dans chaque régione ont varié entre, 0,01 et 0,04 unités OD pour tous les quatre antigènes. La réactivité de 14 des serum positifs à certains antigènes, mais pas à tous les quatre antigènes semi-synthétiques, indique que le porteur et le lieur pourraient être associés à cette réactivité de fond. Il serait justifié de chercher à remplacer les lieurs et porteurs. Nous concluons que la réactivité de fond aux antigènes semi-synthétiques représentant la molécule PG-I de *M. leprae* est de 0,7–1,3% basée sur 0,16 comme valeur positive. A partir de ces données nous avons conclu que la réactivité chez les individus non lépreux était assez basse pour justifier l'usage de ces antigènes dans les opérations de diagnostic, telles que le dépistage des contacts de l'entourage et les populations très endémiques. Lorsque l'incidence et la fréquence de la lèpre sont basses, l'emploi de ces antigènes pour les tests ne se justifie pas économiquement, sauf chez les individus à haut risque. Le dépistage sérologique avec ces antigènes pourrait être utile pour la détection et l'identification de la rechute bactérienne, les réactions de type 1 ou 2, la détection précoce de la lèpre, et le suivi du traitement dans les régions d'endémie.

La evaluacion de cuatro antigenos *Mycobacterium leprae* semi-sinteticos con sueros de populaciones sanas en zonas endemicas y no endemicas

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Resumen Para poder determinar la frecuencia de ocurrencia de los anticuerpos en los antígenos semi-sintéticos de *Mycobacterium lepræ* en populaciones clínicamente sanas que no son pacientes, y para establecer una 'línea de base' para comparar las frecuencias de anticuerpos en los pacientes con antecedentes de lepra y sus contactos, se realizaron ELISAs usando sueros representativos de dos zonas: una de lepra endémica, Cebu City, Islas Filipinas, y una zona no endémica, Chicago, Illinois, EE.UU. Se probaron estos sueros por medio de ELISA IgM indirecta, para la presencia de anticuerpos que reaccionan con cuatro antígenos semisintéticos basados en el antígeno fenólico glicolípido I de *Mycobacterium lepræ*: ND-O-BSA (disacárido natural con enlace octílico con albumina sérica bovina), NT-O-BSA (trisacarido natural con enlace octílico con albumina sérica bovina), ND-P-BSA (disacarido natural con enlace fenólico con albumina sérica bovina), y NT-P-BSA (trisacarido natural con enlace fenólico con albumina sérica bovina). Usando una lectura OD $\geqslant$0,16 como positiva, el antígeno con la seroactividad de fondo más bajo fue ND-O-BSA que reaccionó con 5/398 (1,3%) de los sueros de Cebu, y 3/426 (0,7%) de los sueros de Chicago. Un total de 10 (2,5%) de los 398 sueros de la zona endémica reaccionó con al menos un antígeno y 5 (1,3%) de los sueros reaccionaron con los cuatro antígenos semi-sintéticos. De los 426 sueros de Chicago, 12 (2,8%) eran reactivos con al menos un antígeno y 3 (0,7%) reaccionaban con los cuatro antígenos semi-sintéticos. Los valores medios ELISA para los 22 sueros positivos de cada antígeno variaban entre 0,17 y 0,3 unidades OD, y los promedios para todos los sueros en cada zona variaban entre 0,01 y 0,04 unidades OD para todos los antígenos. La reactividad de 14 de los sueros positivos con algunos de los antígenos, pero no todos los antígenos semi-sintéticos, indica que el portador y los enlaces pueden ser asociados con esta reactividad de fondo. Se justifica la investigación de otros portadores y enlaces. Concluimos que la reactividad de fondo no específica con los antígenos semi-sintéticos que representa la molécula PG-I de *Mycobacterium lepræ* es 0,7 a 1,3%, valor basado en un valor de corte de $\geqslant$0,16 OD. Estos datos nos permiten concluir que la reactividad de individuos libres de lepra fue suficientemente bajo para justificar el uso de estos antígenos en un ambiente diagnóstico, por ejemplo el control de contactos familiales y en populaciones muy endémicas. Cuando la incidencia y frecuencia de la lepra son bajas, pruebas que usan estos antígenos no serían rentables, al menos que se les aplicará a individuos muy expuestos a riesgo. El control serológico por medio de estos antígenos podría ser útil en la detección y diferenciación de los relapsos bacteriológicos, las reacciones de tipo 1 o 2, la detección temprana de la lepra y para controlar el tratamiento en zonas endémicas.