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Seroepidemiological Study on 724 Household Contacts of Leprosy Patients in French Polynesia Using Disaccharide-Octyl-BSA as Antigen[1]

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One of the main problems of leprosy control is the spread of bacilli from infectious subjects before diagnosis ([15]). Therefore, tests for the early diagnosis of the diffuse form of leprosy without clinical symptoms are greatly needed. The most important recent advance in this matter is the development of immunological tests for recognizing *Mycobacterium leprae* infection.

Antigen sharing across mycobacterial species is extensive ([7, 10]). A phenolic glycolipid-I (PGL-I) derived from *M. leprae* has been described which is present in large amounts in the bacillus and surrounding tissue ([11–13]). Antibodies to PGL-I are found in sera from leprosy patients but not in sera from normal individuals or patients infected with other mycobacteria ([2, 5, 19]). Humans respond predominantly with IgM immunoglobulins ([5, 20]). The dominant epitope in this antibody response resides in the terminal sugars of the trisaccharide ([21]). The recent synthesis of this carbohydrate residue and its inherent terminal mono- and disaccharide conjugated to a protein ([4, 9]) provides a very convenient antigen for field application ([3]).

A large population-based study in The Philippines from 1935–1950 and in Burma from 1964–1975 showed that attack rates in contacts of lepromatous cases are 3.3 to 7.5 times higher than those in noncontact individuals ([16]).

More recently, in Micronesia ([8]) serological studies using *M. smegmatis* as antigen showed that 70% of new cases belonging to a contact population have high levels of antibodies from 3 months to 2 years before the clinical onset of the disease.

In French Polynesia leprosy is endemic, and the detection rate per year is 8.8/100,000 inhabitants. Since 1984, a surveillance program of the household contact population has been underway. An ELISA method with the disaccharide moiety of PGL-I coupled to bovine serum albumin (BSA) as antigen was chosen for the serological study of 724 subjects. We report here the results obtained after 2 years of observation.

MATERIALS AND METHODS

Material

Control sera. Positive control sera were collected, before treatment, from 13 multibacillary (4 borderline lepromatous or BL, 9 lepromatous or LL) and 19 paucibacillary (8 indeterminate or I, 5 borderline tuberculoid or BT, 6 tuberculoid or TT) Polynesian leprosy patients.

Negative control sera were collected from 37 individuals from a nonendemic country (France), 68 healthy and presumed noncontact Polynesian blood donors, and 22 proven cases of active pulmonary tuberculosis.

Household contact population. The studied group involved 724 household contacts of proven leprosy cases on Tahiti Island. Each individual is identified by civil data, date of blood collection, relationship with index case, and identification of the index case (detection date, classification, treatment and follow up).

New cases of leprosy detected in French Polynesia during the period 1967 to 1986, according to type (56% paucibacillary and 44% multibacillary) and age, are reported in Table 1. During the same period, 58 relapse cases of multibacillary leprosy were observed.

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TABLE 1. *Type and age of new cases of leprosy detected in French Polynesia from 1967–1986.*

Year	Totals			<15 yrs of age			≥15 yrs of age		
	Total	Pauci-bacillary	Multi-bacillary	Total	Pauci-bacillary	Multi-bacillary	Total	Pauci-bacillary	Multi-bacillary
1967	7	3	4	0	0	0	7	3	4
1968	12	10	2	3	2	1	9	8	1
1969	7	0	7	0	0	0	7	0	7
1970	6	1	5	0	0	0	6	1	5
1971	16	9	7	4	3	1	12	6	6
1972	7	3	4	1	1	0	6	2	4
1973	10	3	7	3	0	3	7	3	4
1974	10	4	6	2	1	1	8	3	5
1975	19	13	6	3	3	0	16	10	6
1976	9	3	6	1	0	1	8	1	7
1977	9	1	8	0	0	0	9	1	8
1978	12	9	3	3	1	2	9	8	1
1979	20	11	9	1	1	0	19	10	9
1980	10	7	3	0	0	0	10	7	3
1981	10	6	4	0	0	0	10	6	4
1982	12	8	4	1	1	0	11	7	4
1983	24	15	9	3	2	1	21	13	8
1984	12	8	4	2	1	1	10	7	3
1985	11	11	0	3	3	0	8	8	0
1986	14	8	6	3	3	0	11	5	6
Totals (%)	237 (100)	133 (56)	104 (44)	33 (14)	22 (9)	11 (5)	204 (86)	111 (47)	93 (39)

The main characteristics of the contact population studied are shown in Table 2. Sera were collected from 1984 to 1986. In 1985, 23% of the population was related to an index case treated for <5 years. Among them, 8% of the population was related to a multibacillary case and 15% to a paucibacillary case. The study of this population involved a clinical examination and a serological test for antibody detection.

Antigen. The antigen used in this study was the natural disaccharide (3,6 dimethyl glucopyranosyl–2,3 dimethyl rhamnopyranosyl) coupled with an octyl spacer arm to bovine serum albumin (ND-O-BSA; Department of Microbiology, Colorado State University, Fort Collins, Colorado, U.S.A.) in a ratio of 7.17 μg sugar to 57.4 μg BSA.

Methodology

Diagnosis of patients. Diagnosis is based on clinical examinations, including examinations of the skin and the large nerve trunks, supplemented by biological tests: the lepromin intradermal reaction, the search for acid-fast bacilli in the nasal mucosa and skin (earlobe and skin lesions), biopsy for pathological examination (P. Destombes and P. Ravisse, Institut Pasteur, Paris) and, since January 1980, biopsy for mouse inoculation and drug-sensitivity testing. These examinations permit the diagnosis of leprosy and the classification of the form of leprosy according to Ridley-Jopling ([14]) and, finally, assignment into paucibacillary or multibacillary categories.

ELISA. The method described by Cho, *et al.* ([5]) was applied with minor modifications.

Plate preparation. Antigen ND-O-BSA was diluted to 16.6 ng sugar/ml in carbonate buffer, pH 9.6, and coated at 50 μl/well to U-bottom Immulon TM2 microplates (Dynatech, Alexandria, Virginia, U.S.A.). Nonspecific binding of sera was measured by a control plate coated with BSA equivalent to that in the antigen wells.

All of the plates were incubated overnight in a moist chamber at 37°C, washed four times with PBST (phosphate buffered saline, pH 7.2, with the addition of 0.1% Tween 20), and blocked for 2 hr at 37°C with PBST containing 5% BSA. The excess BSA was removed, and the plates kept ready for use for 5 days at 4°C wrapped in aluminum foil.

TABLE 2. *Main characteristics of the contact population.*

Index population (type of leprosy)	Total	Age of contact population					
		<15 yrs			≥15 yrs		
		Total	Male	Female	Total	Male	Female
Multibacillary	66%	26%	11%	15%	40%	18%	22%
Paucibacillary	34%	12%	6%	6%	22%	9%	13%
Totals	100%	38%	17%	21%	62%	27%	35%

ELISA protocol. Fifty μl of serum diluted to 1/250 in PBST-5% BSA was added to duplicate antigen and control wells, incubated for 1 hr at 37°C, and washed four times with PBST. Goat anti-human IgM (μ chain specific) peroxidase conjugated reagent (Biosys, Compiègne, France) was introduced at 1 μg/ml in PBST for 1 hr at 37°C. The plates were washed five times and reacted with 50 μl 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) with the addition of H_2O_2 solution at 0.013% final concentration. After 30 min at 37°C, the reaction was stopped by 50 μl of 2.5 N H_2SO_4.

The optical density (OD) was read at 492 nm in a micro-ELISA reader (Titertek, Flow Laboratories, Helsinki, Finland) connected to a microcomputer (Goupil G4, Créteil, France) for automatic calculations of ΔOD = mean OD ND-O-BSA − mean OD BSA.

Reference sera diluted to 1/250 (two positive lepromatous controls and one negative control) were included in each series. Absorbance values were required to range within the intervals of ΔOD = 1.700 ± 10% for positive sera and ΔOD = 0.100 ± 20% for negative controls.

A serum was considered "positive" when the ΔOD exceeded by 2 standard deviations the mean ΔOD obtained from normal Polynesian controls at the same dilution.

Statistics. The activity of each group of sera was expressed by mean ΔOD ± 2 S.E.M. or mean ΔOD ± t 0.05 S.E.M. when the number of sera was <30. The chi-square test was used to compare the frequency distribution of the different groups studied as compared to healthy Polynesians.

RESULTS

Activity of control sera. The activity of healthy Polynesian controls was significantly higher than that of nonendemic country controls ($\alpha < 0.001$). To take into account parameters peculiar to the studied country, such as social and sanitary conditions or ethnic group, the level of antibodies of the healthy Polynesian population was used to calculate the positive cut-off line of the reaction, except when otherwise stated.

Multibacillary patients displayed a very high level of IgM anti-ND-O-BSA ($\alpha < 0.001$) compared to paucibacillary patients whose activity was not significantly different from that of the healthy Polynesians ($\alpha > 0.50$) (Table 3). Considerable overlap was observed in individual values of paucibacillary patients, normal Polynesians, and Polynesian tuberculosis patients.

The detection rate according to the positive threshold defined above (ΔOD cut-off time = 0.806) is 100% (13/13) for the multibacillary group and 5% (1/19) for the paucibacillary group. All of the active pulmonary tuberculosis patients and the negative controls from the nonendemic country were negative; whereas 3/68 (4.5%) of the normal Polynesians were positive.

Serological activity and clinical status of contact population. The mean ΔOD for the 724 subjects was 0.405 ± 0.20 (Table 4). A frequency distribution (The Figure) compares the leprosy contact population to normal Polynesian controls and normal French controls. The serological activity of the contact population was significantly higher than that of the noncontact population ($\alpha = 0.05$).

Ninety-three out of the 724 subjects (12.8%) were detected as seropositive, and 8/724 (1.1%) had an antibody level equiv-

TABLE 3. *Activity of control sera. Mean level of IgM anti-ND-O-BSA and percentage of positive sera.*[a]

Group tested	No. sera	Mean ΔOD ± 2 S.E.M.	S.D.	% Positive	α Value/ Polynesian control[b]
Multibacillary patients (4 BL, 9 LL)	13	1.748 ± 0.138	0.251	100%	<0.001
Paucibacillary patients (8 I, 5 BT, 6 TT)	19	0.391 ± 0.134	0.301	5%	>0.50
Tuberculosis patients	22	0.191 ± 0.080	0.177	0	=0.05
Negative controls from nonendemic country	37	0.109 ± 0.040	0.120	0	<0.001
Healthy Polynesian controls	68	0.310 ± 0.060	0.248	4.5%	

[a] Positive threshold = mean ΔOD + 2 S.D. normal Polynesian = 0.806.
[b] α Value obtained by chi-square test, as compared to healthy Polynesians.

alent to the mean ΔOD − 2 S.E.M. of the multibacillary patients. Two years later, one of this last group (1/8, 12.5%) developed lepromatous leprosy with a bacterial index of 5+ according to Ridley's scale ([6]).

Among the seronegative group (631/724, 87.2%), three of them (3/631, 0.47%) were diagnosed as paucibacillary patients (2 BT, 1 I) between 4 months and 2 years later (Table 5). No serological conversion was observed.

Of the 4 contacts who developed overt disease, 3 were related to a multibacillary and 1 to a paucibacillary index case of whom 3 were treated for <5 years.

The attack rate per year, after 2 years' observation, was 0.27% (4/724/2) in the whole contact population, 0.23% (3/631/2) in the seronegative group, 0.53% (1/93/2) in the seropositive group, and 6.25% (1/8/2) in the highly seropositive group (Table 6).

DISCUSSION

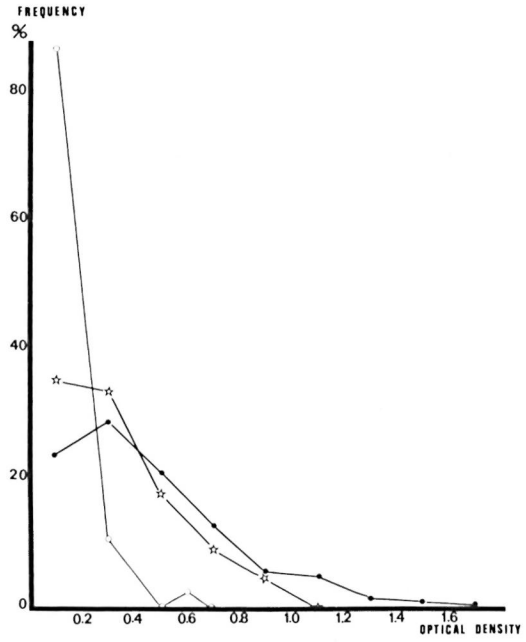
A semi-synthetic glycoconjugate (ND-O-BSA) was used as the antigen to measure IgM antibodies in the control patients, contact population, and healthy subjects.

Normal Polynesians, presumed noncontacts, displayed a higher antibody concentration than did healthy individuals from France. This raises the question of whether or not the reference population should be used to define the threshold of positivity of our assay. Indeed, this antibody elevation may be explained by a polyclonal B-cell activation, for example due to some parasitic diseases such as filariasis, in French Polynesia. Those people also may be exposed to a different range of local atypical mycobacteria that may crossreact with the antigen used. On the other hand, some of them may have effective contact with leprosy patients since our study-field is a small island of 1000 km^2. A similarity of serological frequency distribution between contacts and Polynesian negative controls is rather in favor of these two last hypotheses, subject to confirmation with a larger number of Polynesian and French controls. Indeed, a B-cell activation alone would be expressed by a parallel shift of the Polynesian control curve in the serological frequency distribution in relation to that of the nonendemic controls. Most probably a combination of all three explanations accounts for this antibody elevation, judging from the multiple kinds of sociologic and ethnic groups in the Polynesian population.

TABLE 4. *IgM anti-ND-O-BSA activity of household contact population.*

No. sera	724
Mean ΔOD ± 2 S.E.M.	0.405 ± 0.02
S.D.	0.340
% Positive	12.8[a]
% Positive	49[b]
α Value/Polynesian control	0.05[c]

[a] Positive threshold = mean ΔOD + 2 S.D. normal Polynesian = 0.806.
[b] Positive threshold = mean ΔOD + 2 S.D. normal French = 0.349.
[c] α Value, obtained by chi-squre test, between mean ΔOD values of contact population as compared to healthy Polynesian controls.



THE FIGURE. Serological frequency distribution. Nonendemic negative controls (○) (N = 37); endemic negative controls (☆) (N = 68); leprosy household contacts (●) (N = 724).

Concerning the control sera, detection efficiency was excellent for multibacillary patients (100%) but very weak for paucibacillary patients (5%). Other workers have reported 62% ([5]), 46% ([1]), and 28% ([3]) seropositivity in the paucibacillary group, but they used the values of negative controls from a nonendemic country to define the positive threshold. If we used the same criteria as these workers, we obtained 100% of multibacillary, 42% of paucibacillary, 49% of contacts, and 32% of healthy Polynesian subjects positive to the test. Among the three contacts who developed paucibacillary leprosy, only one of them would be seropositive. So, increasing the sensitivity of the reaction by selecting nonendemic sera as controls results in a larger population to follow up without apparently increasing the leprosy detection rate in the so-defined seropositive group. On the other hand, selection of Polynesian controls increases the specificity of the test and the predictive value for developing a contagious form of the disease. The attack rate in this Polynesian noncontact group was zero during the same period.

Studies on a household contact cohort in an epidemic situation in Micronesia ([17]) showed a detection rate of 8% per year, even though it is around 1% in an endemic area such as Hong Kong ([18]) or Hawaii ([17]). This rate is 0.27% in French Polynesia where leprosy is hypoendemic (8.8/100,000). The annual attack rate in our studied population was found to be 30 times (0.27%) in the whole contact population, 26 times (0.23%) in the seronegative group, and 60 times (0.53%) in the seropositive group higher than in the general population of French Polynesia. But when referred to the group with an antibody level equivalent to that of the multibacillary group, this rate was 710 times higher (6.25%) than the general population, confirming the very high-risk status of those individuals.

Among the four contacts who developed leprosy, three were related to an index case with multibacillary leprosy. The lepromatous patients represent the most important source of infection in the community, and the role of paucibacillary individuals as a source of transmission is not clear at the present stage of our knowledge ([16]), especially since the establishment of the real index case of a contact is not always evident.

TABLE 5. *IgM anti-ND-O-BSA evolution in household contacts who developed clinical leprosy during the study.*

Case no.	Activity as contact	Activity as patient	Interval between 2 sera (mos.)	Contact leprosy type	Index leprosy type
1	0.250 (Neg.)[a]	0.197 (Neg.)[a]	4	BT	M[b]
2	0.708 (Neg.)	0.651 (Neg.)	11	BT	M
3	0.343 (Neg.)	0.330 (Neg.)	23	I	M
4	1.650 (Pos.)	1.833 (Pos.)	24	LL	P

[a] Positive cut-off ΔOD = 0.806.
[b] M = multibacillary; P = paucibacillary.

TABLE 6. *Annual attack rate of leprosy in the household contact population.*

	Whole household contact population	Seronegative group[a]	Seropositive group[a]	
			ΔOD ≥ 0.806[a]	ΔOD ≥ 1.610[b]
No. (%)				
No. registered cases of leprosy in 2 yrs	724 (100%)	631 (87%)	93 (12.8%)[c]	8 (1.1%)
	4	3	1[c]	1
Annual attack rate	0.27%	0.23%	0.53%	6.25%

[a] Positive threshold = mean ΔOD + 2 S.D. normal Polynesians.
[b] Positive threshold = mean ΔOD − 2 S.E.M. multibacillary group.
[c] Includes subjects whose ΔOD ≥ 1.610.

In order to more precisely define the diagnostic or predictive value of this antibody assay, a long-term annual follow up of the contact population is in progress. It involves a clinical examination, a Mitsuda test, and an antibody detection test. Furthermore, a bacteriological examination and a test to detect antigen in sera will be done for the highly antibody-positive subjects.

SUMMARY

A seroepidemiological surveillance of a contact population was started in 1984 in French Polynesia. The ELISA test was used to measure IgM anti-ND-O-BSA in the sera. Specific antibody levels were higher in healthy Polynesians than in normal individuals living in a nonendemic country. The positive threshold of the reaction was fixed according to this background activity in healthy Polynesians. Under these conditions, 100% of the multibacillary patients were detected as seropositive as compared to 5% of the paucibacillary group.

In the population of 724 household contacts tested and observed for 2 years: 93 (12.8%) were seropositive, with 8 (1.1%) showing activity equivalent to multibacillary patients (1 of these 8 individuals developed a lepromatous form of leprosy); 631 (87%) were seronegative and 3 developed a paucibacillary form of the disease (2 BT, 1 I) without any antibody increase. Among those four contacts who developed leprosy, three were related to a multibacillary index case.

These data suggest that this test may be useful for the prediction of multibacillary leprosy. A long-term surveillance of this high-risk population will be able to evaluate the diagnostic and prognostic value of the serological assay.

RESUMEN

En 1984 se inició un estudio seroepidemiológico en una población de contactos en la Polinesia francesa. Se usó la prueba de ELISA para cuantificar los anticuerpos IgM séricos contra el conjugado ND-O-ASB. Los niveles de los anticuerpos específicos fueron más elevados en los polinesios sanos que en los individuos sanos de un país no endémico. El umbral de positividad de la reacción se fijó de aucerdo al valor basal encontrado en los polinesios sanos. Bajo estas condiciones, el 100% de los pacientes multibacilares resultaron seropositivos comparados con el 5% del grupo paucibacilar.

En la población de 724 contactos sanos probados y observados durante 2 años, 93 (12.8%) fueron seropositivos, con 8 (1.1%) mostrando una actividad equivalente a la de los pacientes multibacileres (uno de estos 8 pacientes desarrolló lepra lepromatosa); 631 (87%) fueron seronegativos y de éstos, 3 desarrollaron una forma paucibacilar de la enfermedad (2 BT, 1 I) sin ningún incremento en anticuerpos. Entre los 4 contactos que desarrollaron lepra, 3 estuvieron relacionados con un caso de lepra multibacilar.

Estos datos sugieren que esta prueba puede ser útil para la predicción de la lepra multibacillar. La vagilancia a largo plazo de esta población de alto riesgo permitirá evaluar el valor diagnóstico y pronóstico del ensayo serológico.

RÉSUMÉ

En 1984, on a entrepris la surveillance séroépidémiologique d'une population contact en Polynésie française. L'épreuve ELISA a été utilisée pour mesurer les taux IgM anti-ND-O-BSA dans le sérum. Les taux d'anti-corps spécifiques étaient plus élevés chez des Polynésiens en bonne santé que dans les individus normaux vivant dans un pays non-endémique. Le seuil fixé pour qu'une réaction soit considérée comme positive a été déterminé en se basant sur les résultats observés chez les Polynésiens en bonne santé. Dans

ces conditions, on a constaté que 100% des malades multibacillaires étaient séropositifs, alors que 5% des malades paucibacillaires livraient des valeurs positives.

Dans une population de 724 domiciliaires étudiés et observés pendant 2 ans, on a relevé des résultats séropositifs chez 93 d'entre eux (12, 8%), dont 8 (1, 1%) présentaient une activité équivalente à celle des malades multibacillaires (1 de ces 8 sujets a développé une maladie du type lépromateux); 631 de ces sujets étaient séronégatifs (87%) et 3 ont développé une maladie du type paucibacillaire (2 BT, 1 I) sans aucune élévation des titres d'anticorps. Parmi les 4 contacts qui ont développé la lèpre, 3 étaient en relation avec un cas index multibacillaires.

Ces données suggèrent que cette épreuve peut être utile pour prédire la lèpre multibacillaire. Une surveillance prolongée des populations à haut risque pourrait permettre d'évaluer la valeur diagnostique et pronostique de cette épreuve sérologique.

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