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Use of Anti-*M. leprae* Phenolic Glycolipid-I Antibody Detection for Early Diagnosis and Prognosis of Leprosy[1]

Frantz Agis, Pascal Schlich, Jean-Louis Cartel, Claude Guidi, and Marie-Anne Bach[2]

The phenolic glycolipid-I (PGL-I) of *Mycobacterium leprae* elicits in *M. leprae*-infected humans an antibody response predominantly of the IgM type, exclusively directed against the terminal trisaccharide portion of the molecule, which is specific to the *M. leprae* species ([4, 5, 11, 14, 19]). ELISAs have been developed to detect anti-PGL-I antibodies in leprosy patients and in patients suffering other mycobacterial diseases. The results published so far indicate that these assays indeed detect *M. leprae*-specific antibodies, the levels of which are correlated to the bacterial loads ([1, 5, 7, 16, 19]). Recent works have shown that the native PGL-I molecule can be replaced by semisynthetic antigens comprising the terminal disaccharide, covalently coupled to bovine serum albumin (BSA), with the same—or even better—sensitivity and specificity ([9, 10]).

The possibility of detecting *M. leprae*-specific antibodies opens new perspectives for the early diagnosis of incubating leprosy cases. Studies have been undertaken to follow groups of domiciliary contacts of leprosy patients ([7, 8]). The first results indicate that these subjects do indeed harbor low but significant amounts of anti-PGL-I antibodies, in a proportion largely exceeding the incidence of leprosy among the populations of contacts ([7, 8]). On the other hand, a few of them exhibit high antibody titers and may have increased risks of developing leprosy ([8]). A parallel assessment of cellular immunity by the Mitsuda test should help the prognostic evaluation of the *M. leprae* infection among contact populations.

Indeterminate leprosy also represents an early stage of *M. leprae* infection with an immunological status not yet well defined ([18]). Although these leprosy patients are all considered as paucibacillary patients and treated like tuberculoid patients, some of them most probably are prelepromatous cases, with a deficient cellular immunity and perhaps an underestimated bacterial load, and should receive specific care for the detection of relapse.

Using as antigen the natural disaccharide-octyl bovine serum albumin (ND-O-BSA) ([9]), we have measured anti-*M. leprae* antibodies among the untreated leprosy cases of Guadeloupe, West Indies, and among the domiciliary contacts of these patients. Comparing the results of the seroassay to those of the Mitsuda test by factorial analysis allowed the delineation of several subgroups among the contacts and indeterminate leprosy cases which might have some relevance to the prognosis of their infection.

MATERIALS AND METHODS

Subjects. A group of 62 newly diagnosed leprosy patients [14 lepromatous leprosy (LL) and borderline lepromatous (BL), 19 tuberculoid (TT) and borderline tuberculoid (BT), and 29 indeterminate] classified according to Ridley-Jopling's criteria ([18]) entered this study. They were followed at the community clinic for Hansen's disease in Guadeloupe, West Indies, and were studied before they received any treatment.

A group of 109 intradomiciliary contacts of these patients and a group of 51 healthy Guadeloupean controls (blood bank donors

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[2] F. Agis, D. Pharm., Laboratoire d'Immunologie Cellulaire, Institut Pasteur, BP 484, 97165 Pointe à Pitre, Guadeloupe. P. Schlich, Ph.D., and J.-L. Cartel, M.D., Institut National de Recherche Agronomique, Guadeloupe. C. Guidi, M.D., Dispensaire anti-Hansenien, Guadeloupe. M.-A. Bach, M.D., D.Sci., Unite de Pathologie de l'Immunité, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France.

Reprint request to Dr. Bach.

TABLE 1. *Increased efficiency of anti-ND-O-BSA antibody detection by the use of an anti-human IgG antibody.*

Subjects	Serum dilution			
	1/50		1/250	
	Anti-human IgG		Anti-human IgG	
	−	+	−	+
Healthy controls (N = 18)	322 ± 150[a]	217 ± 102	99 ± 57	59 ± 33
Paucibacillary patients (N = 24)	528 ± 364 (1.6)[b]	408 ± 303 (1.9)	124 ± 199 (1.3)	179 ± 196 (3.0)
Multibacillary patients (N = 14)	1303 ± 224 (4.0)	935 ± 163 (4.3)	880 ± 548 (8.9)	617 ± 241 (10.4)

[a] Mean $\Delta OD \times 10^3 \pm$ S.D.

[b] Ratio $\frac{\text{mean } \Delta OD \times 10^3 \text{ of patients}}{\text{mean } \Delta OD \times 10^3 \text{ of controls}}$.

without known contact with leprosy patients) were also studied.

Most subjects from each group were tested for both anti-ND-O-BSA antibodies and Mitsuda reactivity, with a few exceptions in whom only one of these parameters could be investigated.

Antibody detection. IgM anti-ND-O-BSA was measured by an ELISA according to the methodology described by Cho, *et al.* ([10]), with minor modifications. The ND-O-antigen was provided by Dr. Chatterjee and Dr. Brennan, Colorado State University, Fort Collins, Colorado, U.S.A. It was used at the concentration of 2 μg/ml. In most cases the sera were prediluted to 1/25 in phosphate buffered saline (PBS), pH 7.4, and were incubated for 15 min at 37°C with a goat anti-human IgG antiserum ("Absorbant RF;" Behring, Marburg, West Germany), to the final dilution of 1/50. Sera were used for the test at two dilutions, 1/50 and 1/250. The absorbance was read at 488–492 nm, and the results were expressed by the difference in optical density (OD) between wells coated with ND-O-BSA and wells coated with BSA alone (ΔOD).

Mitsuda reaction. The Mitsuda reaction was performed with lepromin prepared according to the World Health Organization (WHO) standards ([3]). The test was considered as positive when the diameter of the papule was ≥2 mm.

Statistics. Groups of subjects were compared by variance analysis (Fisher's test) or by the chi-squared text.

A factorial analysis of the results of the antibody assay and the Mitsuda assay was also carried out using the method of analysis of correspondences as described by Benzecri, *et al.* ([2]) with a computer program written by F. Tekaïa, (Unité d'Informatique Scientifique, Institut Pasteur de Paris). For that purpose, the ΔODs recorded at the 1/50 and 1/250 serum dilutions were recoded in 5 and 7 classes, respectively, as follows:

Serum dilution 1/50: class 1 = $\Delta OD \times 10^3 < 220$; class 2 = $220 \leq \Delta OD \times 10^3 < 436$; class 3 = $436 \leq \Delta OD \times 10^3 < 655$; class 4 = $655 \leq \Delta OD \times 10^3 < 869$; class 5 = $\Delta OD \geq 869$ (220 = mean of healthy controls; 436, 655, and 869 = mean of healthy controls plus 2 S.D., 3 S.D., and 4 S.D., respectively).

Serum dilution 1/250: class 1 = $\Delta OD \times 10^3 < 59$; class 2 = $59 \leq \Delta OD \times 10^3 < 133$; class 3 = $133 \leq \Delta OD \times 10^3 < 281$; class 4 = $281 \leq \Delta OD \times 10^3 < 428$; class 5 = $428 \leq \Delta OD \times 10^3 < 577$; class 6 = $577 \leq \Delta OD \times 10^3 < 725$; class 7 = $\Delta OD \times 10^3 \geq 725$ (59 = mean of healthy controls; 133, 281, 428, 577, and 725 = mean of healthy controls plus 2 S.D., 6 S.D., 10 S.D., and 14 S.D., respectively).

The analysis of correspondences was performed in a first step on the three well-defined populations of the healthy controls and the tuberculoid and lepromatous leprosy patients. In a second step, indeterminate leprosy patients and contacts were then introduced and analyzed by reference to the first three groups.

TABLE 2. *Anti-ND-O-BSA antibody levels and seropositivity rates among healthy controls, contacts, and leprosy patients.*

Subjects	No.	Serum dilution			
		1/50		1/250	
		Mean ΔOD $\times 10^3 \pm$ S.D.	% positive (>436)	Mean ΔOD $\times 10^3 \pm$ S.D.	% positive (>133)
Healthy controls	51	220 ± 108	4	59 ± 37	2
Contacts	109	261 ± 149	13	105 ± 73[a]	31[b]
Indeterminate leprosy	24	405 ± 312[b]	33[b]	185 ± 198[b]	42[b]
TT/BT leprosy	16	408 ± 224[b]	31[c]	135 ± 91[a]	44[b]
LL/BL leprosy	14	935 ± 169[b]	100[b]	617 ± 250[b]	100[b]

[a] Significantly different from healthy controls, $p < 0.05$.
[b] Significantly different from healthy controls, $p < 0.001$.
[c] Significantly different from healthy controls, $p < 0.01$.

RESULTS

Effects of anti-human IgG antibody on sensitivity of anti-ND-O-BSA antibody assay. The background fixation to control BSA-treated plates varied from one subject to another and represents a cause of lower sensitivity and increased irrelevant variability of the assay. Rheumatoid factors, which we often found in the sera of leprosy patients (data not shown), contribute to this phenomenon ([6, 12, 17, 20]). Our preliminary experiments were performed on a few sera from normal subjects, paucibacillary patients, and multibacillary patients to evaluate the effect of adding anti-human IgG antibodies on the detection of IgM anti-ND-O-BSA antibodies. Such a procedure not only diminished the nonspecific fixation to the BSA-coated control plates but also, and to a higher extent, the fixation of the ND-O-BSA-coated test plates, thus reducing the ΔOD in all groups of subjects. However, such an effect was significantly more pronounced in normal subjects as compared to patients, the final result being a better discrimination between patients and healthy controls, especially when the 1/250 serum dilution was used (Table 1). For further assays, test sera were diluted in buffer containing anti-human IgG antibodies ([20]).

Anti-ND-O-BSA antibody levels and seropositivity rates among controls, contacts, and patients. As seen in Table 2, tuberculoid, lepromatous, and indeterminate leprosy patients exhibited significantly higher antibody levels than did control subjects without known contact with leprosy patients. The lepromatous patients displayed the highest mean antibody level, whatever the serum dilution tested. Contacts of leprosy patients significantly differed from controls only at the 1/250 dilution of the sera.

A threshold of positivity was defined as the mean of the healthy controls plus two standard deviations (2 S.D.). All lepromatous patients were positive at any serum dilution. For contacts and paucibacillary patients, the highest positivity rate was seen at the 1/250 dilution, reaching 31%, 42%, and 44% for contacts, indeterminate, and tuberculoid patients, respectively. Very few subjects were negative at the 1/250 dilution and positive at the 1/50 dilution (2 controls, 2 contacts, and 1 indeterminate leprosy case). The cumulative percentage of subjects seropositive at the 1/50 and/or the 1/250 dilution then reached 6% for healthy subjects, 33% for contacts, and 54% for indeterminate leprosy patients.

Figure 1 shows that the distribution of antibody levels among patients with indeterminate leprosy differs from that seen in patients suffering from tuberculoid leprosy, with a significant proportion of the former displaying high antibody amounts, reaching a level only attained by lepromatous patients.

Influence of age on anti-ND-O-BSA antibody levels. No significant influence of age upon antibody level could be detected among lepromatous patients, tuberculoid patients, or healthy subjects. A positive correlation ($r = 0.46$, $p < 0.05$) was observed among indeterminate patients between anti-

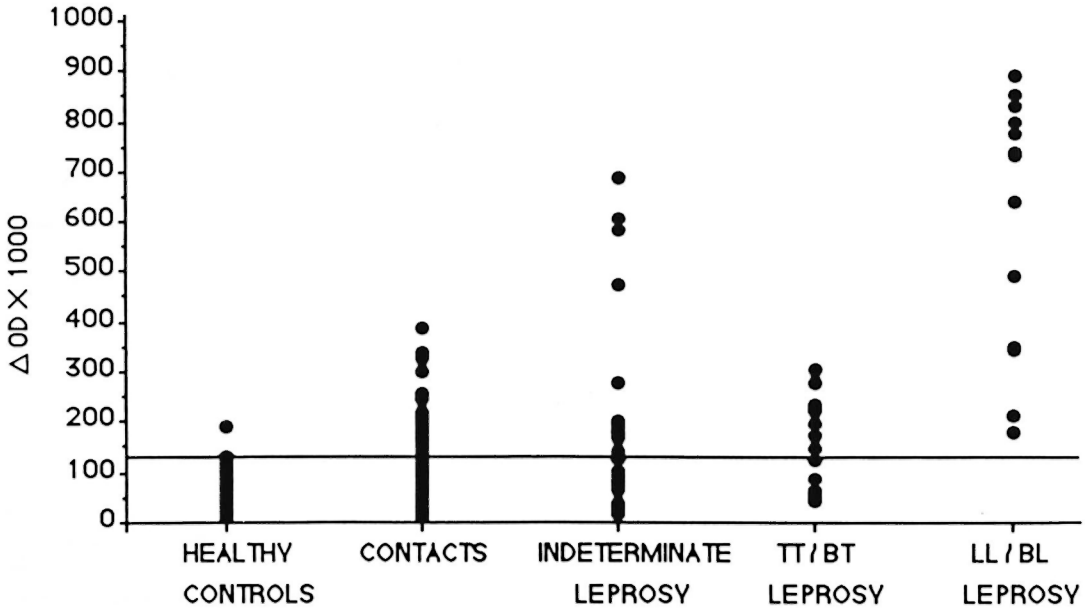

FIG. 1. Anti-ND-O-BSA antibodies in leprosy patients, their contacts, and healthy controls. Sera were tested at the 1/250 dilution. Results are expressed as $\Delta OD \times 10^3$ (absorbance measured at 488–492 nm). Each point represents one individual. Seropositivity cut-off (mean of controls + 2 S.D.) is shown by the horizontal line.

ND-O-BSA antibody levels and age, the oldest patients displaying the highest values. Among contacts, the correlation of antibody levels with age was weak ($r = 0.05$); nevertheless, a significant difference in seropositivity ($p < 0.03$) was observed between children under 15 (18%) and adults over 40 (44%) (Table 3).

Influence of leprosy form of index case on anti-ND-O-BSA antibody levels of domiciliary contacts. Contacts were subdivided into two groups according to the leprosy form of the index case (paucibacillary or multibacillary patients). At the serum dilution of 1/250, the contacts of multibacillary patients exhibited a significantly higher seropositivity rate and higher mean ΔOD than contacts of paucibacillary patients (43% vs 25% and 127 vs 94, respectively; $p < 0.05$) (Table 4).

Distribution of responses to the Mitsuda test. The tuberculoid leprosy patients were all Mitsuda positive and the lepromatous leprosy patients were all Mitsuda negative. Among the healthy controls and contacts of patients, a very large majority were found to be Mitsuda positive (96% and 94%, respectively). The percentage of Mitsuda-positive subjects was significantly less (62%) among patients suffering from indeterminate leprosy. In this group, the percentage of Mitsuda-positive subjects was found to be significantly lower after the age of 40 ($p < 0.05$) (Table 5).

Relationship of anti-ND-O-BSA antibody level to Mitsuda status. Most (but not all) subjects could be tested for both the Mitsuda reaction and the antibody assay. Factorial correspondence analysis allowed us to study the dependency relations between the different modalities of these two variables among the various populations considered. Table 6 shows the distribution of leprosy patients, contacts, and healthy controls according to Mitsuda status and anti-ND-O-BSA antibody levels (seronegative = ≤mean of controls + 2 S.D. for both serum dilutions; seropositive "+" = seropositive at the 1/250 dilution but with $\Delta OD \times 10^3$ remaining equal or below 428 (that is, the mean of controls + 10 S.D.), or seropositive at the 1/50 dilution only; seropositive "++" = seropositive at the 1/250 dilution with $\Delta OD \times 10^3 > 428$ and seropositive at the 1/50 dilution). Six modalities of immune status to *M. leprae* were then defined, from the lowest resistance stages (Mitsuda-negative, seropositive ++) to the highest re-

TABLE 3. *Influence of age on seropositivity.*

Age (yrs)	% of seropositivity in				
	Healthy controls	Contacts	Indeterminate leprosy	TT/BT leprosy	LL/BL leprosy
<16	—	18	11	50	100
		(33)[a]	(9)	(4)	(1)
16–40	3	37	50	25	100
	(29)	(46)	(6)	(4)	(4)
>40	0	44[b]	63[b]	57	100
	(21)	(25)	(8)	(7)	(9)

[a] Number of subjects is given in parentheses.
[b] Significantly different ($p < 0.05$) from age group <16.

sistance status (Mitsuda-positive, seronegative).

The healthy controls represented a homogeneous group of Mitsuda-positive, seronegative subjects. The contact and tuberculoid patient populations shared some characteristics: distribution in two major populations (Mitsuda positive, seronegative, and Mitsuda positive, seropositive +), but the latter status was more frequent among tuberculoid patients than among contacts. A few contacts were Mitsuda negative, with 25% of them showing seropositivity, thus displaying a "lepromatous-like" immune status to *M. leprae*. The lepromatous patients, all Mitsuda negative, also segregated into two groups according to their antibody level.

The indeterminate leprosy patients exhibited a great heterogeneity, since all modalities of immune response to *M. leprae* could be observed (including one Mitsuda-positive subject with high antibody levels). The dominant group was that of Mitsuda-positive, seronegative subjects (37.5%) but, at the opposite, 25% displayed lepromatous-like immune responses to *M. leprae*. The indeterminate leprosy patients also included a significant proportion of subjects (16.5%) who did not show any detectable immune response to *M. leprae* (Mitsuda negative, seronegative), a pattern which was not encountered in any other patient group and was only found in two healthy subjects and in one of the contacts. The indeterminate patients belonging to these different subgroups strongly differed by their age at the time of diagnosis. As seen in Figure 2, old age is associated with a lepromatous-like profile of immune response to *M. leprae*; whereas young patients tend to show better resistance to the bacillus.

DISCUSSION

One objective of the present work was to determine the technical conditions which provided the best sensitivity and specificity of the ELISA detecting anti-ND-O-BSA antibodies.

The better discrimination between paucibacillary patients (that is, subjects displaying presumably low antibody titers) and healthy controls from the same area was obtained with the 1/250 dilution of the se-

TABLE 4. *Influence of bacillary load of index leprosy case on seropositivity of contacts at the 1/250 dilution.*

Subjects	% positive	Mean ΔOD $\times 10^3 \pm$ S.D.
Contacts of paucibacillary patients	25% (N = 72)	97 ± 57
Contacts of multibacillary patients	43%[a] (N = 37)	127 ± 93[a]

[a] $p \leq 0.05$ as compared to contacts of paucibacillary patients.

TABLE 5. *Results of Mitsuda test among healthy controls, contacts, and leprosy patients.*

Subjects	No.	% positive
Healthy controls	48	96
Contacts	69	94
Indeterminate leprosy	29	62[a]
TT/BT leprosy	15	100
LL/BL leprosy	14	0[a]

[a] Significantly different ($p < 0.001$) from all other groups.

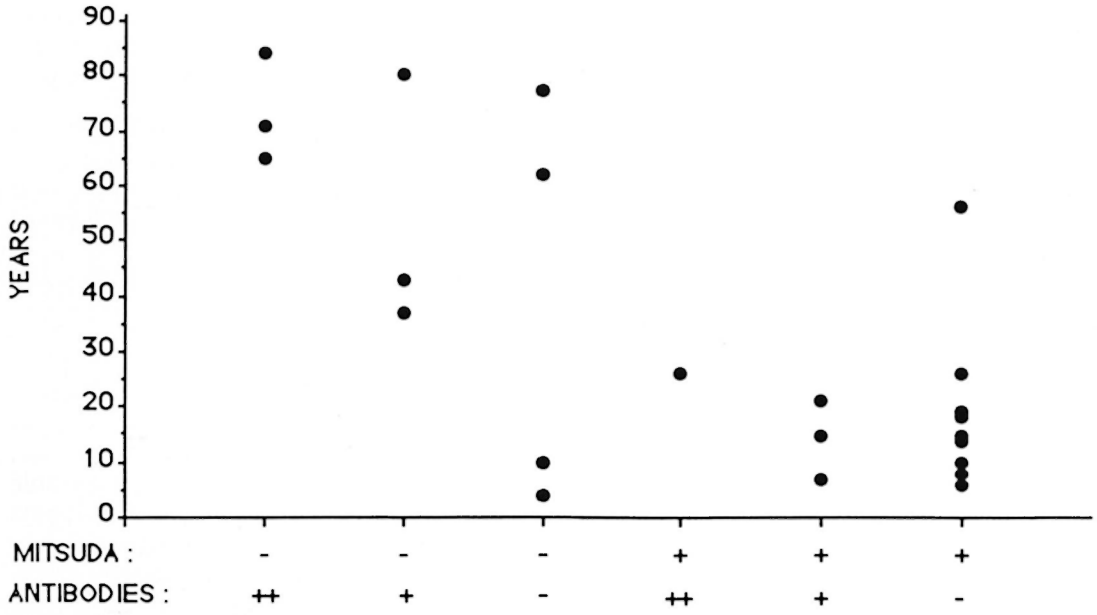

FIG. 2. Age of indeterminate leprosy patients according to their immune responsiveness to *M. leprae*. A Mitsuda assay and anti-ND-O-BSA antibody measurement were performed on the same patients who where then classified into six groups according to their Mitsuda status and antibody level. Mean age is significantly different among these groups ($p < 0.02$).

rum, rather than the 1/50 dilution, due to the high nonspecific background binding observed at the 1/50 dilution among healthy controls. A further improvement of sensitivity was obtained by adding anti-human IgG antibodies to the serum dilution buffer. Indeed, several workers have shown that detection of antigen-specific IgM in whole serum was often impaired by the binding of nonspecific or specific IgG and/or rheumatoid factors ([6, 12, 17]). As shown by Ziegelmaier, *et al.* ([20]), these interferences were partly reduced by the addition of anti-human IgG antibodies to the dilution buffer.

Under these conditions the seropositivity rate reached 42% for BT/TT patients versus only 6% among healthy controls. These results are in agreement with those reported by Cho, *et al.* using either the native PGL-I molecule or the natural disaccharide ([10, 11]). Using ND-O-BSA as antigen, we previously reported a lower percentage of seropositivity among paucibacillary patients from Tahiti ([8]). At variance with the present study, background levels of IgM fixation to ND-O-BSA were relatively high among Tahitian healthy controls and were higher than background levels among controls from nonendemic areas, which was not the case in the present study (data not shown). One should also stress that the small number of paucibacillary patients studied likely affects to a certain extent the evaluation of seropositivity rates in this group.

Among the contact population, the average seropositivity rate was 31%, a percentage similar to that reported by Buchanan, *et al.* ([7]) but, again, higher than that previously found by us among Tahitian contacts ([8]), and also higher than that observed by Dandekar, *et al.*, in India ([13]). As already discussed, in the Tahitian and Indian studies the reference control population was composed of healthy subjects living in the same area as the contacts, where they may have been submitted to various infectious and parasitic agents that can induce a polyclonal B-cell stimulation and nonspecifically increase background levels.

In the present study, contacts of multibacillary patients were found seropositive more frequently than contacts of paucibacillary patients. One can note in this study, as in others, that the percentage of seropos-

itive contacts greatly exceeds the prevalence rate of leprosy in the contact population ([7, 8, 13]), suggesting that a large majority of contacts develop subclinical self-healing *M. leprae* infections.

Another objective of the present work was to evaluate the use of serology for the prognosis of *M. leprae* infection in association with an assessment of cellular immunity as realized by the Mitsuda test. As expected, tuberculoid leprosy patients were all Mitsuda positive and lepromatous patients were all Mitsuda negative. On the other hand, both lepromatous and tuberculoid patients showed some heterogeneity of anti-ND-O-BSA antibody levels, allowing us, on the basis of factorial correspondence analysis, to subdivide each of these categories into two subgroups. As we reported earlier ([1]), these subgroups did not coincide with the Ridley-Jopling classification into borderline and polar forms (data not shown). In addition to the bacillary load, other individual factors most likely influence the amount of anti-*M. leprae* antibody produced. Indeed, we observed in the experimental model of murine infection by *M. lepraemurium* that the level of antimycobacterial antibody produced varied strongly according to the mouse strain studied for a similar bacillary load ([15]).

A large majority of the healthy subjects were seronegative (as expected from subjects without known contacts with leprosy patients) and Mitsuda positive. Mitsuda positivity might have been favored by previous contacts with other mycobacteria (BCG vaccination is compulsory in French territories), and indicates that most subjects of this area possess the intrinsic capacity to develop an efficient cellular immune response toward killed *M. leprae*. It is therefore surprising to note that a third of the contacts of leprosy patients harbor detectable amounts of anti-ND-O-BSA antibodies, which means that the *M. leprae* infection has developed enough in these subjects to elicit a detectable antibody production. One can imagine that these subjects are subjected to repeated exposures to *M. leprae* and/or that living *M. leprae* possess some capacity to partly escape cellular immunity. In terms of immune status, this population of contacts does not differ from that of tuberculoid patients. This raises the intriguing question of the physiopathological mechanisms responsible for the cutaneous and neurological lesions that develop in tuberculoid leprosy patients.

A small minority of contacts was found to be Mitsuda negative and seropositive, an immune status resembling that of lepromatous patients. The follow up of the contact population will ascertain whether or not these subjects represent a high-risk group for the development of lepromatous leprosy.

Indeterminate leprosy represents an early stage of the disease. As paucibacillary patients, patients with indeterminate leprosy are treated according to the protocols designed for tuberculoid leprosy. Our study, however, shows that a fourth of them displayed immune responses of the lepromatous type to *M. leprae*, with some subjects developing very high antibody levels only seen otherwise in lepromatous leprosy. This may result from an individual predisposition to produce high antibody amounts, as discussed earlier, but may also mean that the bacillary load has been underestimated. It is interesting to note that these subjects are significantly older than the others. Age-associated immunodepression may have played a role in this inability to develop an efficient cellular immune response to the bacillus. Further studies are needed to decide whether this population of "lepromatous-like" indeterminate leprosy patients should receive special care.

Only 17% of indeterminate leprosy patients are really "indeterminate" in terms of their immune status (that is, Mitsuda negative and seronegative). Such an immune profile was rarely found among healthy subjects, whether or not they had contact with leprosy patients (1.5% and 4%, respectively). Whether their immune status predisposes Mitsuda-negative subjects to develop leprosy after contact with the bacillus, or whether the contact with the living bacillus can induce a state of specific immunodepression in some subjects formerly Mitsuda positive, remains an unanswered question.

SUMMARY

Untreated patients suffering from tuberculoid, lepromatous and indeterminate leprosy, their domiciliary contacts, and healthy

TABLE 6. *Relationships between Mitsuda test results and anti-ND-O-BSA antibody levels among healthy controls, contacts, and leprosy patients.*

Mitsuda	Anti-ND-O-BSA antibody level[a]	Subjects				
		Healthy controls (N = 48)	Contacts (N = 69)	Indeterminate leprosy (N = 24)	TT/TL leprosy (N = 12)	LL/BL leprosy (N = 14)
−	++	0% (0)	0% (0)	12.5% (3)	0% (0)	71.5% (10)
−	+	0% (0)	4% (3)	12.5% (3)	0% (0)	28.5% (4)
−	−	4% (2)	1.5% (1)	16.5% (4)	0% (0)	0% (0)
+	++	0% (0)	0% (0)	4.5% (1)	0% (0)	0% (0)
+	+	6% (3)	32% (22)	16.5% (4)	58% (7)	0% (0)
+	−	94% (43)	62.5% (43)	37.5% (9)	42% (5)	0% (0)

[a] ++ = $\Delta OD \times 10^3 > 428$ at the 1/250 dilution and > 436 at the 1/50 dilution; + = $\Delta OD \times 10^3 > 133$ and < 428 at the 1/250 dilution, or < 133 at the 1/250 dilution but > 436 at the 1/50 dilution; − = $\Delta OD \times 10^3 \leq 133$ at the 1/250 dilution and ≤ 436 at the 1/50 dilution.

controls, all living in Guadeloupe, West Indies, were tested by an ELISA for detecting IgM antibodies to the terminal disaccharide of the phenolic glycolipid-I antigen of *Mycobacterium leprae*. On most subjects, a Mitsuda test was also performed. A large majority of the tuberculoid patients and healthy subjects were Mitsuda positive. The seropositivity rate reached 44% among tuberculoid patients, and 6% among healthy subjects, with low antibody levels. Lepromatous patients were all Mitsuda negative and seropositive, with antibody production varying from low levels, as seen in tuberculoid patients, to much higher levels.

Indeterminate leprosy patients included 62% Mitsuda-positive subjects and 54% seropositive subjects with a large dispersion of antibody levels. Comparing the results of the Mitsuda test to those of the ELISA by factorial analysis allowed us to define several subgroups among this population: some (25%) showed a "lepromatous-like" immune status (Mitsuda negative, seropositive); others (54%) exhibited "tuberculoid-like" profiles (Mitsuda positive without antibodies or with low antibody levels). "Lepromatous-like" cases were significantly older than "tuberculoid-like" patients. A group of subjects (17%) was Mitsuda negative and seronegative, thus displaying a true "indeterminate" immune profile, which had not been seen in other forms of the disease and had been observed in only 2 out of 51 healthy controls.

A large majority of contacts was Mitsuda positive, with 33% of them being seropositive, indicating that the prevalence of *M. leprae* infection greatly exceeds that of overt leprosy in this population. Only a few contacts displayed lepromatous-like immune responses to *M. leprae* (Mitsuda negative, seropositive) or exhibited an "indeterminate" pattern (Mitsuda negative, seronegative).

RESUMEN

Los sueros de pacientes sin tratamiento afectados de lepra tuberculoide, lepra lepromatosa, o lepra indeterminada, así como los sueros de sus contactos domiciliares y de controles sanos, todos ellos habitantes de Guadalupe en las Indias Occidentales, se probaron por un ensayo inmunoenzimático (ELISA) para buscar la presencia de anticuerpos IgM contra el disacárido terminal del glicolípido fenólico-1 del *Mycobacterium leprae*. También se hizo la prueba de Mitsuda en la mayoría de los pacientes. La gran mayoría de los pacientes tuberculoides y de los sujetos sanos fueron Mitsuda positivos. El grado de seropositividad alcanzó el 44% entre los pacientes tuberculoides y el 6% entre los sujetos sanos, con bajos niveles de anticuerpo. Los pacientes lepromatosos fueron todos Mitsuda negativos y seropositivos, con títulos de anticuerpos que oscilaron de valores bajos como los observados en pacientes tuberculoides, a valores mucho mayores. Los

pacientes con lepra indeterminada incluyeron 62% de sujetos Mitsuda positivos y 54% de sujetos seropositivos con una gran dispersión de niveles de anticuerpo. Comparando los resultados de la prueba de Mitsuda con los del ELISA por análisis factorial se pudieron definir varios subgrupos entre esta población: algunos (25%) mostraron un estado inmune "parecido al lepromatoso" (Mitsuda negativos, seropositivos); otros (54%) exhibieron un perfil "parecido al tuberculoide" (Mitsuda positivos sin anticuerpos o con muy bajos niveles de anticuerpo). Los casos "parecidos a lepromatosos" fueron significativamente más viejos que los "parecidos a tuberculoides." Un grupo de sujetos (17%) fue Mitsuda negativo y seronegativo, es decir verdaderamente indeterminado. Este perfil inmune "indeterminado" no se había observado en otras formas de la enfermedad y sólo se encontró en 2 de 51 sujetos sanos.

La gran mayoría de los contactos fueron Mitsuda positivos con 33% de ellos seropositivos. Esto indica que la prevalencia de la infección con *M. leprae* excede grandemente la prevalencia de la enfermedad abierta en esta población. Solo unos cuantos contactos exhibieron una respuesta "parecida a la lepromatosa" (Mitsuda negativos, seropositivos) o "indeterminada" (Mitsuda negativos, seronegativos).

RÉSUMÉ

On a procédé à une épreuve ELISA chez des malades atteints des formes tuberculoïde, lépromateuse, et indéterminée de la lèpre, tous non traités, de même que chez leurs contacts domiciliaires, et chez des sujets témoins sains, en Guadeloupe, en vue de déceler des anticorps IgM au disaccharide terminal de l'antigène PLG-I de *Mycobactérium leprae*. On a également pratiqué une épreuve de Mitsuda chez la plupart des sujets. Une large majorité des malades tuberculoïdes et des sujets sains étaient Mitsuda positifs. Le taux de séropositivité atteignait 44% chez les malades tuberculïdes, et 6% chez les sujets sains, avec des taux faibles d'anticorps. Les malades lépromateux étaient tous Mitsuda négatifs et séropositifs, la production d'anticorps variant entre des valeurs faibles, telles qu'on peut les voir chez les tuberculoïdes, à des valeurs beaucoup plus élevées.

Parmi les malades atteints de lèpre indéterminée, 62% étaient positifs, et 54% témoignaient d'une séropositivité, les taux d'anticorps présentant des valeurs très dispersées. L'analyse factorielle comparant les résultats de l'épreuve de Mitsuda avec ceux de l'ELISA a permis de distinguer plusieurs sous-groupes dans cette population. Quelques uns, 25%, témoignaient d'un statut d'immunité semblable à celui de la lèpre lépromateuse (Mitsuda négatif et séropositivité); d'autres (54%) montraient des profils analogues à la lèpre tuberculoïde (positivité du Mitsuda, sans anticorps ou avec des taux faibles d'anticorps). Les cas ressemblant aux malades lépromateux étaient significativement plus âgés que les malades présentant une ressemblance avec les sujets tuberculoïdes. Un groupe d'individus (17%) présentait une épreuve de Mitsuda négative alors qu'ils étaient également séronégatifs, ce qui constitue une manifestation réelle d'un profil immun "indéterminé" qui n'a pas été relevé dans les autres formes de la maladie, mais qui a cependant été observé chez deux individus parmi les 51 témoins en bonne santé.

La grande majorité des contacts étaient positifs au Mitsuda, 33% d'entre eux étant également séropositifs, ce qui indique que la prévalence de l'infection par *M. leprae* dépasse largement la prévalence de la lèpre clinique dans cette population. Quelques contacts seulement ont livré des réponses à *M. leprae* ressemblant aux "type lépromateux" (Mitsuda négatif, séropositivité), ou un profil "indéterminé" (Mitsuda négatif, séronégativité).

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