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Risk and Protective Factors for Leprosy Development Determined by Epidemiological Surveillance of Household Contacts▽

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Household contacts of leprosy patients are the group with the highest risk of developing the disease, and although many risk or prevention factors have been identified, they have not been employed in leprosy-monitoring programs. This investigation aimed to establish the relative risks or the preventive effects of the presence of BCG vaccination, the Mitsuda test, and the ML-Flow assay. Household contacts (1,396) were monitored for a 5-year period. Twenty-eight contacts (2%) developed leprosy and had their clinical and operational classifications established. All immunological tests were performed, and intradermal BCG vaccination was given after the BCG scar count. Of the affected contacts, 75% developed the disease in the first year, and 71.4% were classified as having paucibacillary forms. Contacts of lepromatous leprosy patients presented a 3.8-fold-higher risk of developing leprosy. BCG vaccination and the Mitsuda test showed a protective effect against leprosy of 0.27 (at least one scar) and 0.16 (>7 mm), respectively, and the positive ML-Flow test indicated a relative risk approximately sixfold higher for occurrence of the disease. All unfavorable combinations of two and three assays generated significant risk values that ranged from 5.76 to 24.47, with the highest risk given by the combination of no BCG scar, negative Mitsuda test, and positive ML-Flow test. We suggest that the BCG vaccination may be given to stimulate Mitsuda test positivity, reducing the patient's risk of developing multibacillary forms. The high significance of these tests may have a great impact on programs to monitor contacts and should be used to improve early detection and treatment.

Leprosy is a curable disease with well-defined etiology, but better diagnostic tools and therapeutic strategies are lacking, which, together with the sociocultural prejudice, become important obstacles to overcome for early detection and protection of the susceptible population, especially for the household contacts of leprosy patients, who should be the priority of disease control programs in order to interrupt transmission and reduce physical and social disabilities.

Untreated multibacillary (MB) patients are probably the most important source of *Mycobacterium leprae* transmission. It is estimated that household contacts of MB patients have a relative risk of developing leprosy that is 5- to 10-fold greater than that of the general population (9, 12, 24). However, in many areas, the number of MB patients is very small, and they may not represent the most important source of infection (14). There is increasing evidence that subclinical transmission may occur, since even in countries where leprosy is highly endemic, for many patients, no history of close contact with a leprosy patient can be established (12).

If risk factors for leprosy occurrence can be established, adherence of patients to appropriate treatment and monitoring of their household contacts may become easier, thus enabling early diagnosis.

Household contacts, due to their proximity to leprosy patients, are the group with the highest risk of developing the disease, but their risk factors are not well defined in leprosy-monitoring programs, frequently characterized by a late diagnosis and maintenance of the disease transmission chain.

Several groups recently used postgenomic approaches to discover new antigens for leprosy diagnosis (1, 20, 22, 23). All those studies explored sequences for the identification of *M. leprae*-specific proteins or peptides that may be suitable for the serodiagnosis of different stages of leprosy disease. However, the prospective antigens relevant to the diagnosis of the disease must be validated experimentally.

In some countries, like Brazil, the cell-mediated immune response to *M. leprae* has been measured by the intradermal injection of a suspension of heat-killed bacillus, also known as the Mitsuda test (16). Measurement of the local intradermal reaction at the injection site in leprosy patients is an important indicator of efficient cellular immunity to *M. leprae* and a good prognosis. Lepromatous patients often do not show an intradermal reaction in response to the bacillus. At the opposite end, tuberculoid patients display a strong positive reaction. Therefore, in the majority of healthy individuals, positive reactions are associated with a lower risk of developing the disease (13).

Bacillus Calmette-Guérin (BCG) vaccination has been extensively applied to humans, and the use of its intradermal injection for leprosy prevention has been proposed (18), but results are controversial (2, 3, 6, 7, 15). Many reports have confirmed its interference with the Mitsuda response, stimulating positivity (10, 11, 27). The presence of BCG scars has been associated with some protection against the development

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of the disease. It has been suggested that two or more BCG scars may reduce the incidence of the disease, especially the MB forms (6, 7, 19).

Recently, a simple, robust, and rapid lateral-flow test for the detection of immunoglobulin M antibodies to the *M. leprae*-specific phenolic glycolipid 1 (PGL-1) was proposed for use in the operational classification of leprosy patients into paucibacillary (PB) and MB patients as well as the identification of contacts at a high risk of developing leprosy (5).

The aim of this investigation was to characterize important risk factors associated with leprosy incidence in household contacts in order to support monitoring programs with the use of screening procedures that might identify individuals at a high risk, improving early diagnosis and treatment.

Leprosy occurrence risks were estimated for the Mitsuda test, BCG vaccination, and ML-Flow assays (anti-PGL-1 detection) in household contacts that were divided into two groups: affected and healthy individuals.

MATERIALS AND METHODS

A study was performed at the National Reference Center of Leprosy and Sanitary Dermatology of the Clinics Hospital, Federal University of Uberlandia, Uberlandia, Brazil, to investigate risk factors in 1,396 household contacts of leprosy patients seen from 2002 to 2007. The protocol was approved (protocol no. 025/2000) by the Federal University of Uberlandia Research Ethics Committee, and informed consent was obtained from each contact individual.

Data collection. The variables studied were the number of affected and nonaffected contacts according to the operational classification and clinical forms of their index cases, the Mitsuda test, the ML-Flow assay, the BCG scar count, and the year of disease onset after diagnosis of the index case.

Soon after diagnosis of the index case, the tests were performed in all household contacts and subsequently once in a year. However, we used only the results of first test for the risk estimates. The ML-Flow assay was repeated after diagnosis of the affected contacts.

Mitsuda test. The Mitsuda test was performed before BCG vaccination in all contacts. The Mitsuda test was read by an experienced leprosy specialist 28 days after an intradermal injection of 0.1 ml lepromin suspension (6.0×10^7 bacilli/ml, heat killed, supplied by the Instituto Lauro de Souza Lima, Bauru, Sao Paulo, Brazil) in the upper one-third of the anterior aspect of the right forearm. Results were measured in millimeters for quantitative and qualitative analyses (25). Contacts were divided into two categorical classes according to WHO recommendations (26): "negative" for readings of ≤7 mm, which consisted of negative and weakly positive reactions, and "positive" for readings of >7 mm, which consisted of positive reactions, and strongly positive reactions or with the presence of pustular lesion and/or ulceration.

ML-Flow assay. A serological assay consisting of a lateral-flow test was used in all contacts to detect circulating *M. leprae* anti-PGL-1 antibodies (Kit Biomedical, The Netherlands) provided by The Netherlands Leprosy Relief. The contacts were classified into five subjective categories based on color development, ranging from zero (negative) to 4 (highly positive) (5).

BCG vaccination. The Brazilian Leprosy Control Program has recommended the application of two doses of intradermal BCG to the household contacts of leprosy patients. The second dose should be given 6 months after the first dose (4). The presence of a prior BCG scar was considered to be the first dose, regardless of the vaccination period. After confirming the absence of leprosy, contacts with no scar received two applications of BCG intradermally spaced 6 months apart, while those with a BCG scar received only a single intradermal dose.

Contact follow-up. All contacts of each leprosy patient were monitored every year for 5 years, and all tests and clinical data were collected soon after the diagnosis of the index case and after the onset of the disease in contacts.

Statistical analysis. The results are presented as odds ratios (ORs) from a case control study. A protective effect, defined as 1 − OR, was also shown. Significant confidence intervals (CIs) were established for a 95% probability level.

TABLE 1. Operational and clinical classifications of index cases and household contacts that have developed leprosy

Clinical form[a]	No. (%) of patients		
	PB	MB	Total
Index case			
TT	2 (7.1)	0 (0.0)	2 (7.1)
BT	1 (3.6)	1 (3.6)	2 (7.1)
BB	0 (0.0)	7 (25.0)	7 (25.0)
BL	0 (0.0)	1 (3.6)	1 (3.6)
LL	0 (0.0)	16 (57.2)	16 (57.2)
Total	3 (10.7)	25 (89.3)	28 (100.0)
Affected contacts			
I	1 (3.6)		1 (3.6)
TT	6 (21.4)		6 (21.4)
BT	13 (46.4)		13 (46.4)
BB		6 (21.4)	6 (21.4)
BL		1 (3.6)	1 (3.6)
LL		1 (3.6)	1 (3.6)
Total	20 (71.4)	8 (28.6)	28 (100.0)

[a] TT, tuberculoid; BT, borderline tuberculoid; BB, borderline-borderline (i.e., midborderline between lepromatous and tuberculoid); BL, borderline lepromatous; LL, lepromatous leprosy; I, indeterminate.

RESULTS

During the 5-year period of 2002 to 2007, 367 families with an average of 3.8 household contacts per family were monitored, totaling 1,396 contacts investigated. During this monitoring period, 28 contacts developed leprosy, with an incidence rate of 4 per 1,000 persons per year, including coprevalent cases. Among all individuals, 79% were contacts of MB cases of leprosy.

In the present work, 2% (28 out of 1,396) of the contacts developed leprosy, and 89.3% (25 out of 28) were contacts of the MB index case; 64% (16 out of 25) of these were contacts of lepromatous leprosy (LL) index cases, representing 57.2% (16 out of 28) of all affected contacts (Table 1). Contacts of MB index cases presented a risk that was 2.3-fold higher than that of the contacts of PB index cases (95% CI [$CI_{95\%}$], 0.69 to 7.70), although the difference was not statistically significant ($P > 0.05$). However, household contacts of LL patients presented a 3.8-fold-higher risk of leprosy development than contacts of patients with the other clinical forms ($CI_{95\%}$, 1.77 to 8.06; $P < 0.05$).

Of the affected contacts, 71.4% (20 out of 28) were classified as being PB cases, and only one case (3.6%) was of the indeterminate form. Most affected contacts presented clinical forms of the borderline group (71.4%), and the borderline-tuberculoid (BT) form was the most frequent (46.4% [13 out of 28]) in the PB group. The lepromatous pole showed frequencies of 3.6% (1 out of 28) for each of the borderline-lepromatous (BL) and LL forms (Table 1).

Of the contacts that developed disease symptoms, 75% occurred during the first year of epidemiological surveillance, and two of them were coprevalent cases detected concomitantly with their index cases (Table 2).

As to the intradermal BCG, 25% (7 out of 28) of the contacts developed PB forms (two tuberculoid [TT] cases and five

TABLE 2. Times between index cases and diagnoses of leprosy in affected contacts

No. of yrs after index case until leprosy diagnosis	No. of affected contacts	% of affected contacts
0[a]	2	7.1
1	19	67.9
2	1	3.6
3	2	7.1
4	1	3.6
5	2	7.1
6	1	3.6
Total	28	100.0

[a] Concomitant leprosy detection in contacts together with their index cases (coprevalence).

BT cases) after a BCG vaccine booster; four of these cases were observed after the first dose, and three cases were observed after the second dose. The majority of cases (six out of seven) occurred during the first year of follow-up, with an average time to appearance of leprosy between 5 and 6 months after the BCG dose.

Household contacts with one or more BCG scars (72.9% [997 out of 1,396]) displayed a protective effect of 0.27 ($CI_{95\%}$, 0.13 to 0.59) against the appearance of leprosy in comparison to the affected contacts (57.1% [16 out of 28]) with no BCG scar, which could also be interpreted as an estimated risk of leprosy occurrence that is 3.7-fold higher for contacts with no BCG scar (Table 3).

In reference to the Mitsuda test, which measures the specific cellular immune response against *M. leprae*, 85.7% of the affected contacts (24 out of 28) presented Mitsuda test readings varying from 0 to 7 mm, which means a variation from negative to weakly positive, and only 14.3% (4 out of 28) presented readings from 8 to ≥10 mm, which is considered to be a positive to strongly positive response. The contacts with positive Mitsuda responses (>7 mm) presented a protective effect against the development of leprosy of 0.16 ($CI_{95\%}$, 0.05 to 0.46), which could also be interpreted as an estimated risk of disease occurrence that is 6.25-fold higher for contacts with a Mitsuda result of ≤7 mm (Table 3).

For humoral immune response evaluation, the ML-Flow test detected 39.3% (11 out of 28) of positive results among affected contacts against a positivity of 10.4% (145 out of 1,396) among healthy contacts, representing a relative risk almost six times higher for the appearance of the disease in the positive cases (OR, 5.58; $CI_{95\%}$, 2.56 to 12.15) (Table 3).

The combination of results from the three assays and the occurrence of leprosy in the contacts (Tables 4 and 5) indicated significant relative risks for the unfavorable results, such as the absence of BCG scars, a negative Mitsuda test, and a positive ML-Flow test. All unfavorable combinations of two and three assays generated significant risk values that ranged from 5.76 to 24.47, with the highest risk presented by the combination of no BCG scar, a negative Mitsuda test, and a positive ML-Flow test (Table 4). Importantly, the presence of the positive results for BCG and Mitsuda conferred a protective effect for the occurrence of the disease of 0.06 ($CI_{95\%}$, 0.009 to 0.57), which could also be interpreted as a protection factor that is 17-fold higher than those of contacts with other combinations (Table 4).

TABLE 3. Relative risks for development of leprosy in household contacts of patients with the disease based on the ML-Flow test (anti-PGL-1), Mitsuda test, and BCG scars

Indicator and type of contact[a]	No. of positive results	No. of negative results	OR ($CI_{95\%}$)
ML-Flow test			
Affected	11	17	5.58 (2.56–12.15)
Healthy	142	1,226	
Mitsuda test			
Affected	4	24	0.16 (0.05–0.46)
Healthy	694	674	
BCG scars			
Affected	12	16	0.27 (0.13–0.59)
Healthy	997	371	

[a] For the ML-Flow test, a positive result was a score of ≥1 and a negative result was a score of 0; for the Mitsuda test, a positive result was >7 mm and a negative result was ≤7 mm; for BCG scars, positive was considered to be at least one scar, and negative was considered to be zero scars.

TABLE 4. Leprosy development in household contacts of patients and estimated relative risks based on BCG scars, Mitsuda test, and ML-Flow test (anti-PGL-1)[a]

Presence of BCG scar	Result by:		Affected contacts		Healthy contacts	
	Mitsuda test	ML-Flow	No.	%	No.	%
−	**−**	**−**	7	25.0	206	15.0
+	**−**	**−**	8	28.6	487	35.6
−	+	**−**	1	3.6	123	9.0
+	+	**−**	1	3.6	433	31.7
−	**−**	**+**	8	28.6	22	1.6
+	**−**	**+**	2	7.0	42	3.1
−	+	**+**	1	3.6	11	0.8
+	+	**+**	0	0.0	44	3.2
Total			28	100.0	1,368	100.0

[a] For the ML-Flow test, a positive result was considered to be ≥1 and a negative result was considered to be 0; for the Mitsuda test, a positive was considered to be >7 mm and a negative result was considered to be ≤7 mm; for BCG scars, positive was considered to be the presence of at least one scar and negative was considered to be no scars. Results in boldface type indicate unfavorable results.

TABLE 5. Risk factor combinations based on BCG scars, Mitsuda test, and ML-Flow test (anti-PGL-1)

Risk factor combination	OR	$CI_{95\%}$
BCG (−)/Mitsuda (−)/ML-Flow (+) × all others	24.47	9.7–61.5
BCG (−)/ML-Flow (+) × all others	19.16	8.1–45.5
Mitsuda (−)/ML-Flow (+) × all others	11.30	5.0–25.4
BCG (−)/Mitsuda (−) × all others	5.76	2.7–12.3
BCG (+)/Mitsuda (+) × all others	0.06	0.009–0.57

DISCUSSION

This is an epidemiological study in an area of Brazilian where leprosy is endemic that measured the relative risks of leprosy occurrence and its clinical forms in household contacts over a 5-year period. We used the strategy of evaluating three simple clinical procedures, BCG vaccination, the Mitsuda test, and the ML-Flow assay, to determine the specific risks for the development of leprosy or protective effects against this disease, as these procedures may become promising tools for the identification of susceptible individuals during monitoring of household contacts in leprosy control programs.

The 5-year period of surveillance of household contacts was chosen since it represents the average leprosy incubation period (13). The majority of cases of the disease in contacts (75%) occurred during the first year, including the coprevalent cases, which means that the most important focus in programs for monitoring of contacts must happen in the first year, and a careful analysis is vital in order to identify and protect individuals at a higher risk.

The household contacts of LL patients presented a risk of developing leprosy that was almost four times higher than that of the contacts of patients with other clinical forms, which is similar to the risks reported previously (9, 12, 24). This higher risk is a consequence of the high bacillary load pressure of the LL index case on their contacts and possible familial genetic factors.

BCG vaccination has been associated with the prevention of leprosy since 1939 (11), but its level of protection has remained controversial. However, a meta-analysis (28) using 29 different studies provided convincing evidence of a protective effect, and none of the studies revealed a negative protective effect. The percent summary of a protective effect including all case control studies was 58%, with a CI ranging from 47% to 67%, while in the population investigated in our study, the protective effect was 74%, with a CI ranging from 41% to 87%. Another meta-analysis (21) also presented a general protective effect of 26% in 7 experimental studies and 61% in 19 observational studies.

Moreover, we have found that the BCG scar presented a protective effect of 0.02 against the MB forms, indicating 98% protection, which means that BCG vaccination is an important tool in clinical and epidemiological practice in order to prevent MB forms, the most significant source of transmission. Similar results have also been demonstrated elsewhere previously (7, 17, 21).

Based on our results, it is suggested that an additional intradermal BCG booster dose be maintained in leprosy control programs for household contacts, aiming for protection against leprosy, mainly against MB forms. This work corroborates other reports (6, 7, 19) that were decisive in establishing the adoption of two intradermal BCG doses as a control measure for the household contacts of leprosy patients.

Prior to the BCG vaccination, the Mitsuda specific cellular immunological assay and the ML-Flow test must also be used to identify contacts at a higher risk of developing leprosy. Before the BCG vaccination is given, it is important to perform the Mitsuda test to evaluate the status of the specific cellular immunity to the bacillus, as the BCG vaccine may induce positivity in the Mitsuda test (10, 11, 27).

The Mitsuda test has not been standardized by the WHO for clinical classification or for epidemiological studies, since the WHO system of leprosy classification has been based on PB and MB criteria (26). However, our findings highlight the usefulness of the intradermal application of the whole human lepromin in order to identify individuals at higher risk of developing leprosy. It is well known that the late lepromin reaction is a measure of the individual's ability to generate a cell-mediated immune response to an immunizing dose of *M. leprae* and also a measure of granulomatous hypersensitivity (13). Although the relationship between Mitsuda reactivity and resistance is not yet fully established, it has been clearly demonstrated that the long-lasting late lepromin negativity in areas where leprosy is endemic is associated with an increased risk of developing LL (13).

Our investigation corroborates the view that the Mitsuda reaction is often closely correlated with resistance to *M. leprae* after natural infection, with a protective effect against leprosy of 0.16, which indicates that a negative result ($\leq$7 mm) presents a risk of the disease to occur that is six times higher. Therefore, this cellular response may be an indicator of acquired protective immunity rather than an expression of hypersensitivity in contacts. Hence, we suggest that the application of the Mitsuda test in countries where leprosy is endemic may be an important epidemiological approach for monitoring household contacts of leprosy patients.

In this context, an additional intradermal BCG vaccination may stimulate positive conversion in those contacts with temporary negative Mitsuda responses, reducing their risk of developing MB forms.

Finally, the use of the ML-Flow test is justified due to its good correlation with MB forms (5), and PGL-1-seropositive contacts are also associated with a higher risk of developing leprosy than the seronegative contacts (8), which is also confirmed by our findings that showed a risk that was almost six times higher for those with positive results.

The individual results of each assay in this investigation demonstrated that the positivity for the Mitsuda test ($>$7 mm) and BCG vaccination (presence of a scar) were associated with protection against leprosy in household contacts. On the other hand, the positive ML-Flow result indicated a higher risk of leprosy development. However, the combination of these three assays has never been made before, and ours is the first report using this approach to estimate the relative risks or protection of household contacts against leprosy.

The most important combination includes all three assays, i.e., BCG (negative)/Mitsuda (negative)/ML-Flow (positive), and has generated an estimated relative risk that is almost 25 times higher for the appearance of the disease than the other combinations. The second-highest relative risk value, which was almost 20 times higher for disease development, was observed for the combination of negativity for a BCG scar and a positive ML-Flow test. Because the BCG vaccination has the ability to induce reactivity to the Mitsuda test (10, 11, 27), this may explain the presence of two contacts with positive Mitsuda ($>$7 mm) tests and no BCG scars, which were both household contacts of LL index cases, suggesting that the exposure of these two cases to a higher bacillary load may have induced the Mitsuda positivity.

The combination of positive antibody assays and negative

findings in cellular immunity has been suggested elsewhere previously (13), and it was expected to be associated with a particularly high risk for the MB forms; however, we have found PB-affected contacts more frequently (mostly the BT form, with a Mitsuda result varying from 0 to 7 mm), which may be due to the constant medical assistance in every year during the follow-up, allowing early detection, or it may be possible that the BCG booster induced the clinical outcome of the cellular immune response of the bacillus that was already present in the skin. We have demonstrated that this combination not only is very important in determining the risk but is also interesting in establishing a protocol for monitoring household contacts in leprosy control programs, improving early diagnosis.

Our results have significant implications for epidemiological research and clinical practice, and the use of simple assays for monitoring of contacts may identify high-risk individuals and may also provide protection. Therefore, we suggest the following approaches: (i) household contacts of leprosy patients must be monitored during the first year after diagnosis of the index case; (ii) an additional intradermal BCG booster dose must be given in leprosy control programs for household contacts, aiming for protection against leprosy, mainly against MB forms; and (iii) the use of the combination of the three assays may discriminate individuals at higher risk of developing leprosy from contacts with significant protection factors, which could lead to a closer monitoring program for those at risk as well as a subsidized, new, and effective control strategy for leprosy. This proposal may justify the chemoprophylaxis of close contacts of leprosy patients who fit the highest-risk categories defined in this study.

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