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Immune Response to *M. leprae* of Healthy Leprosy Contacts[1]

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The most important mode of the transmission of *M. leprae* from an infected to a noninfected person still remains uncertain ([4]). Close contact over a prolonged period of time is widely accepted as a required condition, indicating that a direct skin to skin contact is necessary for the transmission of the disease. However, critical reviews of the matter ([1, 9]) favor other routes such as by droplet infection.

Two factors may account for the slow progress in this field. First, the incubation period in leprosy is so long that the tracing of contacts and source of infection is very difficult. Second, no method has up to now been available by which the proportion of any population which has been exposed to *M. leprae*, but does not contract the disease, can be determined.

It is now well-established that lymphocytes may respond by enlargement and cell division when they are exposed to antigens towards which they are sensitized. This phenomenon has been called lymphocyte transformation and is now widely used as an immunological method.

Lymphocyte transformation has recently been established independently in two laboratories as a method of measuring the immune response to *M. leprae* ([3, 5]). By this technic, tuberculoid patients may respond strongly, while lepromatous patients fail to respond. Thus, the method shows correlation with the degree of host resistance in leprosy. Moreover, in the present report it is shown that this technic detects a specific response to *M. leprae* because other mycobacteria, such as *M. tuberculosis*, are only able to provoke relatively very low, if any, response to *M. leprae*. Lymphocyte transformation may therefore reveal itself as a useful method in the study of transmission and susceptibility of leprosy.

A high proportion of positive responses was found among medical personnel working with leprosy and among household contacts of leprosy patients. The results indicate that *M. leprae* is frequently transmitted to contacts of patients including medical attendants. When so few develop the disease, it apparently is due to the emergence of effective immunity in the majority of those who become exposed.

MATERIALS AND METHODS

Test subjects. In this study 94 persons have been tested. Two were tested twice, once before and then after three to six months of leprosy work. Both observations are included in the study. Twenty-six belonged to the staff of the All-Africa Leprosy Rehabilitation and Training Centre (ALERT) and the Armauer Hansen Research Institute (AHRI). Thirty persons were participants in courses held at ALERT. They came from Africa, Europe, Asia and America. A majority of them (21 persons) had been engaged in leprosy work previously. Ten belonged to the Norwegian community in Ethiopia, or were visitors without any known contact with leprosy. Of the medical personnel, 27 were doctors or medical students, 12 were nurses or dressers, 4 physiotherapists and 13 leprosy field workers or other staff. The length of time this group (altogether 66 persons) had worked with leprosy is summarized in Table 1. In addition, 16 household contacts of leprosy patients who accompanied the patients to the hospital were examined. Of these, nine were relatives of the patients, while six were married to the patients, and

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TABLE 1. *Length of time nonhousehold contacts had worked with leprosy patients.*

Length of time worked with leprosy patients (years)	Course participants	ALERT-AHRI staff	Others	Total
0	9	3	10	22
0 – 1	7 (+1)[a]	1 (+1)[a]		8 (+2)[a]
1 – 5	6	15		21
> 5	8	7		15
Total	30 (+1)[a]	26 (+1)[a]	10	66 (+2)[a]

[a] Numbers in parentheses indicate those two who were retested after three to six months of leprosy work.

one was married to the sister of a patient. Twelve nurses and dressers from the tuberculosis training center in Addis Ababa were included in the study for assessment of the specificity of the method. They had been working with tuberculosis for at least four years. Only two had been BCG vaccinated (four and seven years previously).

Lymphocyte transformation. The method of lymphocyte transformation has been described in detail elsewhere ([5]) and is only summarized here. Leukocytes were prepared by dextran sedimentation from defibrinated peripheral blood. The cells were cultivated in ordinary pyrex centrifuge tubes, 2.5×10^6 cells per tube, in 2.5 ml Eagle's medium (BDH) containing 20% human serum from individuals not exposed to leprosy. The experiments were set up in duplicate with two tubes against *M. leprae*, two against BCG and two serving as controls. The amount of antigen used was 1×10^7 bacilli per tube.

After seven days of cultivation at 37°C the lymphocytes were stained with acridine orange and examined for transformation by fluorescent microscopy as described by Lamvik ([6]). The results were recorded as net transformation, i.e., the percentage of transformed cells in cultures containing antigen minus the transformation observed in the cultures without antigen. Up to one percent transformation may occasionally be seen in control cultures.

Statistical analysis. Figure 2 indicates that the percent of transformations of the lymphocytes in the various groups is neither "normally" distributed nor of equal variance so it appeared inappropriate to use the *t*-test to measure significance. Instead, the results were divided into two categories, namely "transformation" present ($\geqq$ 1%) and "transformation" absent ($<$ 1%). The significance of the difference between the proportions of the two categories was measured by the chi-squared test, using graphs prepared by M. V. Mussett, Statistical Services Section, National Institute for Medical Research, London, and based on Mainland's tables ([7]).

RESULTS

The specificity of lymphocyte transformation in response to *M. leprae*. In order to assess the specificity of the reaction, the response to *M. leprae* was compared with that to BCG in two groups. The first consisted of 12 nurses and dressers working at the tuberculosis center (Addis Ababa) and the second of 18 healthy individuals who had not been working with leprosy. The scatter diagram is shown in Figure 1. The first group responded strongly to BCG, on the average in 21.1%, as compared to 3.08% on the average to *M. leprae*. This was a 14.7% cross-reactivity. Since only two in this group had been BCG vaccinated (more than four years previously), we assume that the strong response to BCG, and the possible cross-reactivity to *M. leprae*, was caused by exposure to the human tubercle bacillus.

In the second group at least 12 had been BCG vaccinated. None had been working with leprosy. The average response to BCG was 7.03% as compared to 0.51% to *M. leprae*. This gives an average cross-reactivity of 7.25%. Furthermore, of five responding with more than 10% transformation to BCG, none responded to *M. leprae*.

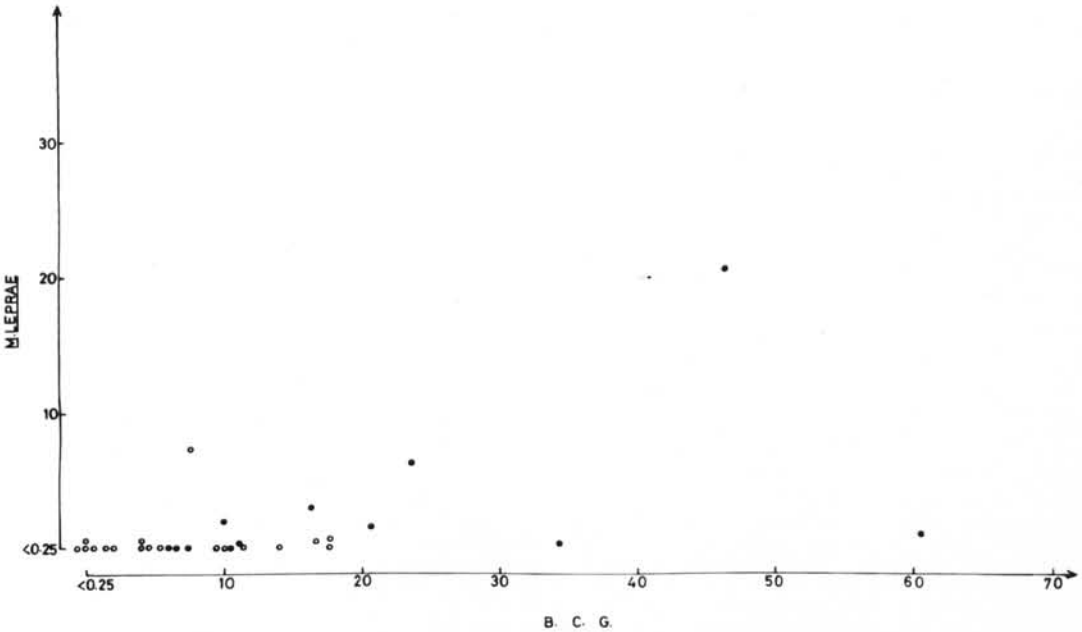

FIG. 1. Scatter diagram of the percent lymphocyte transformation response to BCG as compared to *M. leprae* in 12 staff members at the tuberculosis training center, Addis Ababa (•) and 18 people who had not previously worked with leprosy (o).

Since both groups consisted of individuals living in leprosy endemic areas where they may have been exposed to leprosy, the value of 15% seems to represent the maximal degree of cross-reactivity between *M. tuberculosis* and *M. leprae.*

Lymphocyte transformation response to *M. leprae* related to duration and degree of exposure to leprosy patients. As shown in Figure 2, only one of 22 individuals (i.e. 4.6%) who had not had occupational or household contact with leprosy patients responded (lymphocyte transformation $\geqq$ 1%) to *M. leprae.* This person (of English origin) had lived in Ethiopia for 18 months and visited the physiotherapy department at ALERT several times.

Of the ten persons who had worked with leprosy for less than one year, six responded to *M. leprae.* The difference between these two groups is statistically significant ($P < 0.05$). In three of the six cases the response to *M. leprae* may be explained as caused by a 15% cross-reactivity to *M. tuberculosis.* If these are counted as negative, the difference between the two groups is not significant ($P > 0.05$).

Two persons tested before they began work with leprosy, and three to six months later, converted from negative to weak positive reactions.

Twenty-one people who had worked with leprosy from one to five years were examined. Seventeen responded to *M. leprae* (81%) of which only two cases may be explained as due to *M. tuberculosis* (15/21, i.e. 71% positive response). The difference between this group and the first one which had not worked with leprosy is statistically highly significant ($P < 0.01$).

A very similar proportion of responders was found among 15 people who had worked with leprosy for more than five years (13/15, i.e. 87%). Of the 13 responders 4 could be explained as due to *M. tuberculosis.* If those are counted as negative, 9 of 15 (i.e. 60%) responded to *M. leprae.* The difference between this group and the first one is statistically highly significant ($P < 0.01$).

It is also interesting to note that the strength of lymphocyte transformation seems to be related to degree of contact. Seven workers at the leprosy clinic of Addis Ababa ("Gate clinic") gave an average response of 21.2% to *M. leprae.* They were

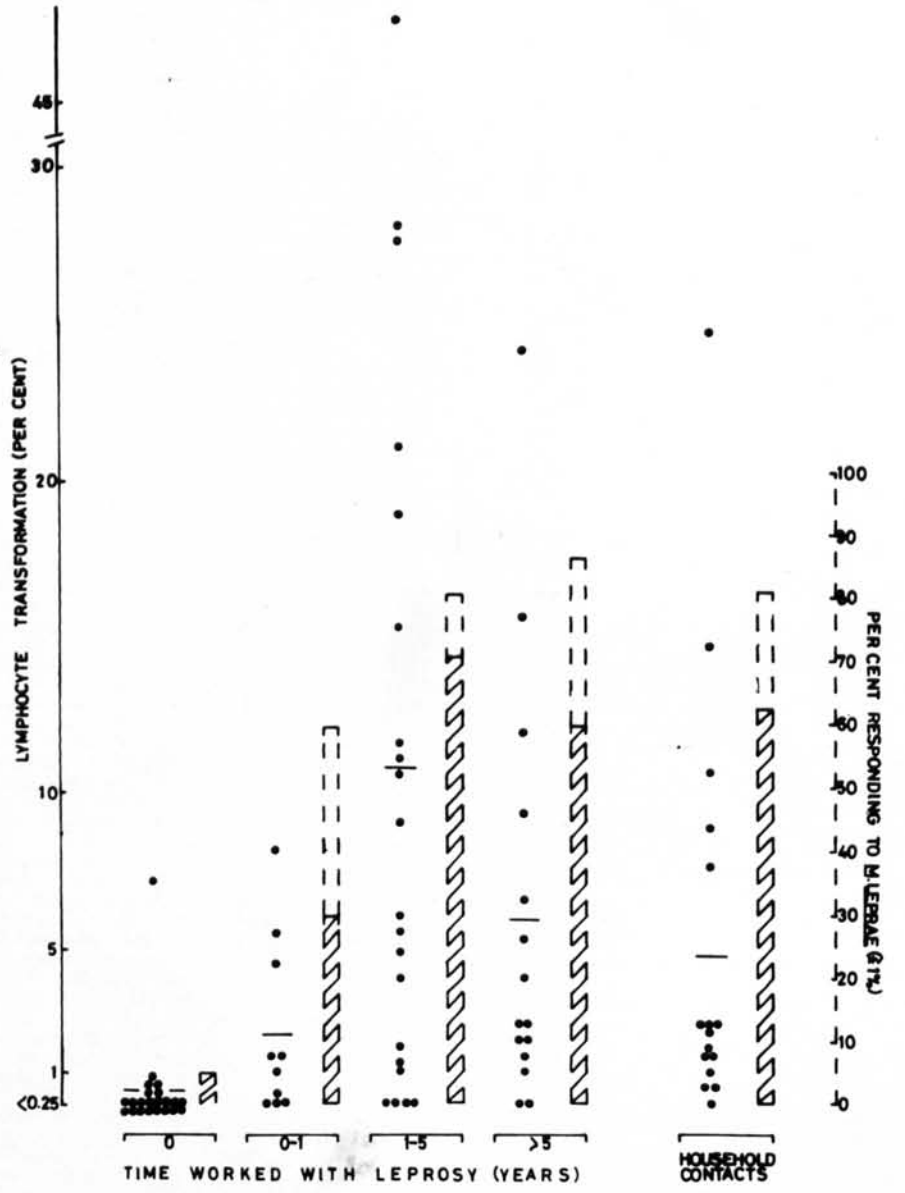

FIG. 2. Lymphocyte transformation response to *M. leprae* related to the length of time subject worked with leprosy and in household contacts of leprosy patients. The results of each individual are shown (•) in reference to the left vertical axis, — = mean of each group. The columns refer to the percentage (right vertical axis) in each group giving a positive response (≥1%). The hatched part of the columns shows the percentage of positive responders, if those whose response to *M. leprae* may be explained as due to a 15% cross-reactivity of *M. tuberculosis* are counted as negative.

daily concerned in examining a number of patients. A comparable group of nine hospital workers, whose patient contact was much less intimate, gave an average of 5.9% lymphocyte transformation response to *M. leprae*.

Lymphocyte transformation to *M. leprae* in household contacts. Among household contacts, 13 of 16 gave a positive response (81%), 3 of which may be explained as due to *M. tuberculosis*. If these are regarded as negative then 10 of 16, i.e. 62.5%,

remained positive. The difference between this group and the first group is statistically highly significant ($P < 0.01$).

DISCUSSION

The lymphocyte transformation test makes it possible to examine some aspects of the immune response to *M. leprae*. Because of the high degree of specificity of the test with respect to *M. tuberculosis*, the maximal degree of cross-reactivity caused by *M. tuberculosis* being on the order of 15%, the method may be used to detect individuals in any population who have been exposed to *M. leprae* and have become immune without contracting the disease clinically.

As shown in this study, approximately 80% of medical personnel will, after one year of work with leprosy patients, respond to *M. leprae*. This suggests that *M. leprae* is frequently transmitted from patients to medical staff including doctors. Although the present study does not reveal the precise route of transmission it would seem unlikely that food and the gastrointestinal tract are an important route of entry, since food is, in general, not consumed at work.

Medical personnel come in direct contact with the patients through their hands. Since even in lepromatous patients bacilli are not found on the surface of their intact skin ([10, 11]), this route of transmission would seem unlikely. By exclusion, we think that an important route by which the bacilli may be transferred to medical personnel is by air droplets. The large amounts of viable bacilli which may be detected in the nasal discharge of lepromatous patients ([16]) support this view.

Since *M. leprae* is transmitted to personnel in a hospital where the hygienic conditions are much higher than among people in leprosy endemic areas, it raises the question to what extent improvement in hygienic conditions can be expected to contribute to the control of leprosy.

This study suggests that the absence of disease in the great majority of medical personnel attending leprosy patients is due to the development of effective immunity. The same would apply to household contacts.

However, the response does not seem to differ in strength from that of tuberculoid patients. The strongest responding group in this study (those who had worked for one to five years with leprosy) gave an average response of 10.68%, while tuberculoid patients (TT and BT combined) gave an average response of 11.12% ([5]). Why do then some become immune without developing disease, while others develop disease despite the same degree of immunity?

In analogy with other infectious diseases, the virulence and size of challenging doses could be critical factors. However, no variation in the virulence has so far been found in mice among 400 strains of *M. leprae* isolated from different parts of the world ([12]). Furthermore, with such a slowly multiplying organism as *M. leprae*, it is likely that even a large challenging dose would quickly be controlled by the much more rapidly developing immune response. More likely, in our view, is that the local concentration of *M. leprae* at which the individual is generating the immune response may be critical. With a challenging dose of equal size and virulence, the immunogenic threshold level of *M. leprae* may be expressed as a function of time after exposure.

The hypothesis shown in Figure 3. If one responds early after exposure (low threshold level) one will become immune. However, if one fails to start to respond before, let us say after two to three years, the bacilli will have had time to settle down and multiply to such an extent that when the immune response is triggered, a lesion will appear where the bacilli are located. If the response is very weak (lymphocyte transformation negative) the lesion may appear as a vague, indeterminate patch (unpublished observations). If the response is strong and the bacilli have had time to multiply in more than a few foci, the patient will appear as TT.

The longer the time taken before the immune response is established, the further down in the Ridley-Jopling scale ([14, 15]) (closer to lepromatous) the patient will fall. Patients who originally appear as BT cases, but go into a reversal reaction ([13]), will clinically remain as BT, but develop a TT histologic pattern. This is indicated in

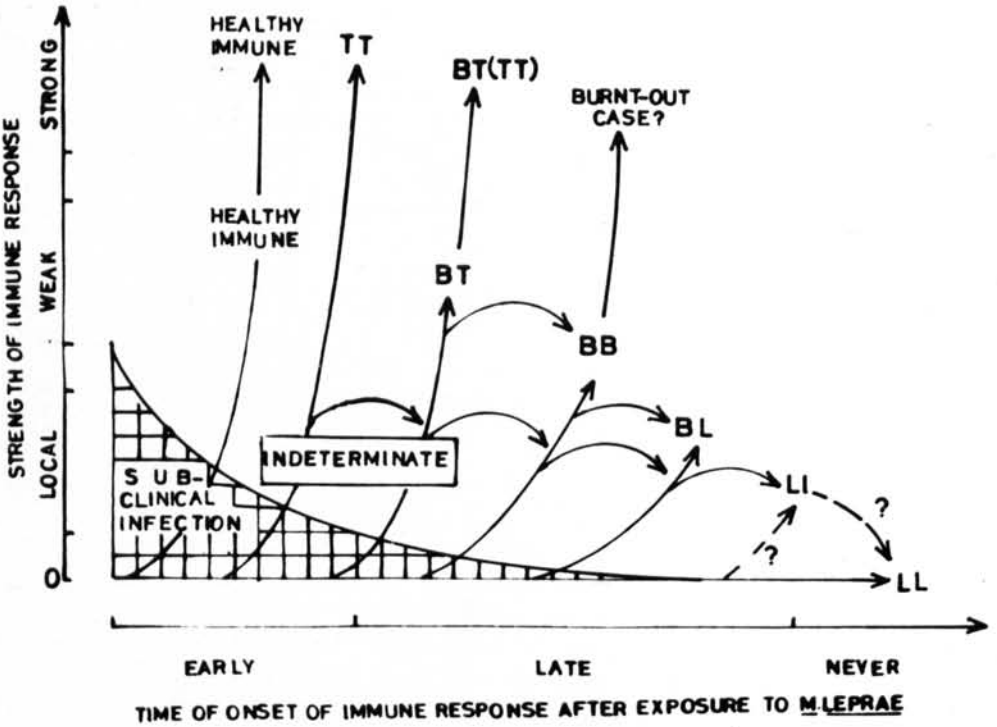

FIG. 3. Schematic representation of the development of various forms of leprosy based on the Ridley-Jopling scale; TT = polar tuberculoid, BT = borderline tuberculoid, BB = borderline, BL = borderline lepromatous, LI = indefinite lepromatous, LL = polar lepromatous and complete immunity as related to the strength of the immune response and time of onset after exposure to *M. leprae*.

Figure 3 as BT (TT). Similarly it is possible that in BB cases the disease may be arrested by a reversal reaction which will cause much tissue damage due to the large antigenic load. Burnt out cases may fit into this category.

"Subclinical infection" in Figure 3 is meant to be the stage at which no sign of disease is apparent nor has any definite immunity yet developed.

It would seem premature to define precisely the strength of immune response in terms of percent lymphocyte transformation test (LTT). However, in Figure 3 "local" means negative in the LTT. With more sensitive technics a response might be detected. "Weak' 'will cover the range from 1%-10%, while more than 10% might be considered "strong." Only when the response has become strong may the disease be arrested. To define the time might be even more difficult, but "early" perhaps covers the first 5 years and "late" from 5-20 years. LL cases fail to respond (i.e. LTT negative) even after many years of treatment when the BI has been reduced to zero (unpublished observations).

The model shown in Figure 3 certainly represents an over-simplification. Any individual perhaps might move up and down in a much more complex pattern than shown in the figure. The factors which influence the movement remain unknown. However, it is interesting to note that a change from indeterminate to tuberculoid lesions after BCG vaccination has been noted ([8]). Since the degree of cross-reaction between *M. leprae* and BCG is so small, this may be due to a nonspecific stimulation of the immune system by BCG rather than due to a specific effect.

If in a given population, a great majority develop tuberculoid (mostly TT) leprosy on exposure, one would expect that BCG vaccination during the incubation period could establish an immune response so ear-

ly that no lesion would appear. This non-specific effect of BCG may have contributed to the protection found in the Uganda trial ([2]).

Factors causing a downgrading shift remain to a large extent unknown, but infectious agents and particular viruses are known to suppress cell-mediated immunity ([18]).

It is interesting to note that since a great majority of those who become exposed to *M. leprae* become immune without contracting the disease, even tuberculoid leprosy would appear to occur in a minority of individuals with a relative deficiency of immune responsiveness to *M. leprae*. This may explain why in several studies cell-mediated immune responses to tuberculoid patients have been found suppressed as compared to control groups ([17]).

SUMMARY

The method of lymphocyte transformation has been used to study the immune response to *M. leprae* among contacts of leprosy patients. It is shown that the method detects an immune response specific for *M. leprae*. The cross-reaction caused by *M. tuberculosis* is maximally in the order of 15%, but probably considerably less.

Only 1 of 22 (4.6%) persons who had not worked with leprosy gave a positive response to *M. leprae* while more than 80% of medical personnel who had worked with leprosy for more than one year gave a positive response. Among household contacts, 13 of 16 (81%) responded to *M.* leprae.

It is concluded that *M. leprae* is frequently transmitted to contacts of leprosy patients including medical personnel. The low prevalence of disease among such contacts appears to be due to the development of effective immunity in a great majority of those who become exposed.

The significance of these findings is discussed in relation to the transmission and pathogenesis of leprosy.

RESUMEN

Se utilizó el método de la transformación de linfocitos para estudiar la respuesta inmunitaria al *M. leprae* entre los contactos de pacientes de lepra. Se demuestra que el método detecta una respuesta inmunitaria específica para el *M. leprae*. La reación cruzada producida por *M. tuberculosis* es de alrededor de un 15% como máximo, pero probablemente es considerablemente inferior.

Solamente 1 de 22 personas (4,6%) que no habían trabajado con lepra dieron una respuesta positiva al *M. leprae*, mientras que más del 80% del personal médico que había trabajado con lepra durante más de un año dió una respuesta positiva. Entre los contactos familiares, 13 de 16 (81%) respondieron al *M. leprae*.

Se concluye que el *M. leprae* se transmite frecuentemente a los contactos de los pacientes de lepra, incluyendo al personal médico. La baja prevalencia de la enfermedad entre tales contactos parece ser debida al desarrollo de una inmunidad efectiva en una gran mayoría de las personas que se encuentran expuestas.

El significado de estos hallazgos se discute en relación con la transmisión y con la patogenia de la lepra.

RÉSUMÉ

La méthode de la transformation lymphocytaire a été utilisée pour étudier la réponse immunitaire á *M. leprae* chez des contacts de malades de la lèpre. On a constaté que cette méthode met en évidence une réponse immunitaire spécifique pour *M. leprae*. La réaction croisée produite par *M. tuberculosis* est á son maximum de 15 pour cent, mais probablement beaucoup moins.

Sur 22 personnes qui n'avaient pas travaillé auprés de malades de la lèpre, 1 seulement soit 4,6 pour cent, a fourni des réponses positives à *M. leprae*, alors que plus de 80 pour cent du personnel médical qui avait travaillé avec des malades pendant plus d'un an, montraient des réponses positives. Parmi les contacts domiciliaires, 13 sur 16, soit 81 pour cent, répondaient á *M. leprae*.

On en conclut que *M. leprae* est fréquemment transmis aux contacts de malades de la lèpre, y compris le personnel médical. La faible prévalence de la maladie parmi ces contacts, semble être dueau développement d'une immunité efficace dans la grande maporité de ceux qui sont exposés. La signification de ces observations est discutée, en relation avec les problèmes posés par la transmission et la pathogénèse de la lèpre.

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Editorial Note. It is worth noting that references 3 and 5 above are not, as may seem to be implied in paragraph 4, page 243, the first reports of lymphocyte transformation abnormalities related to the immune response in leprosy. We regret not having noted this possible discrepancy in interpretation in time to discuss it with the authors before we were in press.—OKS