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Lymphocyte Transformation Test in Healthy Contacts of Patients with Leprosy. I. Influence of Exposure to Leprosy within a Household[1,2]

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The key role of lepromatous leprosy for the propagation of leprosy has been clearly demonstrated in many studies, but the secondary attack rate even in close household contacts of patients with lepromatous leprosy is small under usual endemic conditions ([10, 17, 20]). Patients with tuberculoid leprosy play only a minor role, if any, in the transmission of leprosy. The existence of asymptomatic carriers, postulated on the basis of findings of acid-fast bacilli in the skin of contacts of patients with leprosy, has yet to be proven ([12, 34]).

Aspects of the immune status of contacts of leprosy patients who do not develop the disease have been investigated repeatedly since the introduction of skin tests ([5, 7, 8, 11, 16, 21, 28, 33]), but the results are contradictory and difficult to interpret.

Interest in the immunologic status of contacts of leprosy patients has revived with the introduction of *in vitro* methods of cellular immunology into leprosy research ([3, 14, 18, 25]). Godal and his co-workers ([13, 15]) were the first to apply the lymphocyte transformation test (LTT) to household and occupational contacts of leprosy patients. They interpreted the significantly greater responses to *Mycobacterium leprae* antigen in contacts of patients with leprosy than in controls as a clear indication of subclinical infection among clinically healthy contacts. The findings of these two studies were generally confirmed by Myrvang ([24]) who examined a similar group of people using the leukocyte migration inhibition test. Price *et al* ([28]), comparing children in contact with blood relatives who had leprosy with matched unexposed controls, found no difference in the LTT responses to *M. leprae* antigen but a significantly stronger early lepromin reaction in the children of patients.

In these previous studies the authors did not demonstrate a relationship between the degree of infectiousness of the index patient and the degree of reactivity in the contacts. As the interaction among different factors involved in the etiology of leprosy, especially the risk of exposure to *M. leprae* and the influence of factors inherent in the host, remains a central question in the epidemiology of this disease, it seemed necessary to examine this question more thoroughly.

In our study we have investigated LTT responses to antigens of *M. leprae*, using a more sensitive technic than previously available, in household contacts of tuberculoid and lepromatous patients and in carefully chosen controls in a leprosy-endemic area of Ethiopia. In this paper we describe the influence of the type and duration of exposure to *M. leprae* on the sensitization of the contacts. In a subsequent publication ([23]) we evaluate the influence of various host factors on the LTT response.

POPULATION AND METHODS

Study area and study population. The study was conducted among the Chaha-Gurage who live in the Shoa Province of Ethiopia about three hours drive from Addis Ababa, where the laboratory tests were performed (Fig. 1). The land is semi-mountainous, having an average altitude of 1,700 m and a tropical upland climate. Roads are scarce, and most places can be reached only by mule or on foot.

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The Chaha-Gurage are Semitized people of Cushitic stock. Their culture is based on the cultivation of *Ensete edulis* or "false banana plant" which provides the major source of food. In addition, cattle are kept and some secondary food crops are grown. The standard of living is very low with little variation. Social ties are close, and people gather frequently within households, villages and at markets. The villages contain on the average 150 houses. Households are made up of one to four houses which are located in a common compound together with the ensete field. The houses are large by Ethiopian standards and clean despite generally poor hygienic conditions. The head of the household and his wife and unmarried children live in the largest house, his married sons with their families in the smaller houses. There are usually single relatives and servants living completely integrated within the families.

Leprosy is endemic in the study area. The prevalence rate of registered leprosy patients in Shoa Province has been estimated to be about 2.5 per thousand ([4]). In the study area, 20% of registered patients have the lepromatous type of the disease. The stigma of leprosy is comparatively small, and patients usually continue to live with their families after the onset of clinical disease. Tuberculosis, which is common, and *M. avium* and *M. gordonae* act also as mycobacterial skin sensitizers in this area ([26]). On the other hand, BCG vaccination has not been done extensively. Medical services consist mainly of a mission hospital (Attat Hospital) and an independent outpatient clinic (Gura Mission Station). The governmental Leprosy Control Service operates through the general medical services.

Study groups. A nonleprous patient contact group and a control group were identified consisting of all members of households which were selected on the basis of index persons who had come for treatment to the outpatient clinics of Attat Hospital or Gura Mission Station. A household was defined as consisting of all people who lived in one compound. Requirements for the inclusion of a household into the study were the agreement by the head of the household to all planned examinations, the location of the household within three hours from Attat Hospital, and the presence of at least four household members on whom the complete examination could be expected to be performed. During the period from February 1975 until January 1976, thirty households meeting these criteria were examined. A map showing the geographic location of the households is given in Figure 1.

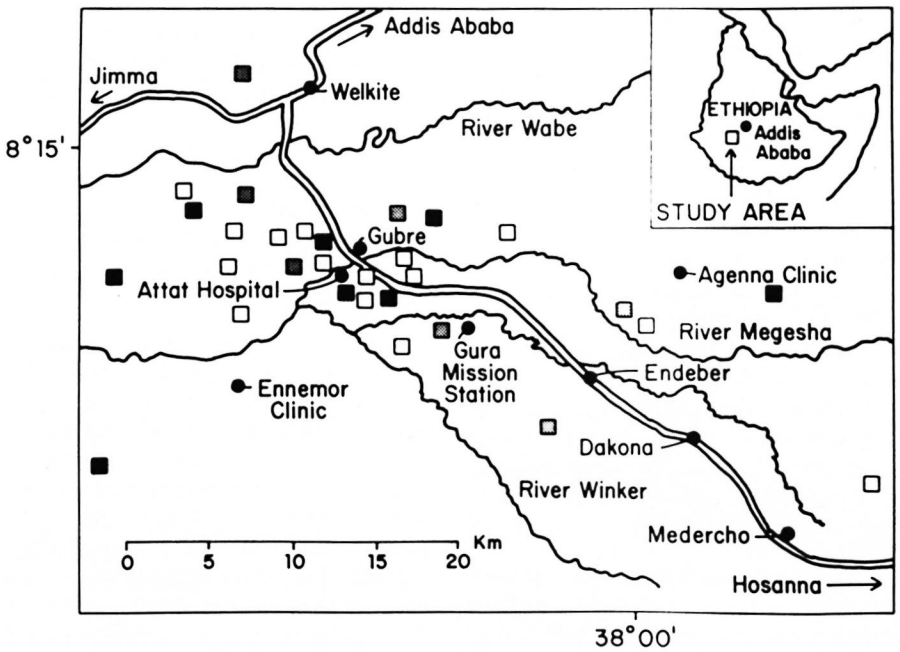

FIG. 1. Study area with approximate location of the households (■ = with a lepromatous patient, ▒ = with a tuberculoid patient, □ = control households).

Index persons with leprosy for the patient contact group. Fifteen patients with a confirmed diagnosis of leprosy who had received regular treatment for less than two years were selected as index patients (12 males, 3 females). They were diagnosed and classified according to the criteria given by Ridley and Jopling ([29,30]), with modifications ([25,31]) on the basis of physical examination, bacteriologic examination of slit skin smears taken from six sites, and histologic examination of a skin biopsy. In addition, a microscopic examination of smears from nasal discharge was performed for acid-fast bacilli (AFB). Information about duration of the symptoms and duration and regularity of treatment was obtained and cross-checked with the clinic records. The ages ranged from 10 to 60 years. Seven of the patients were classified as tuberculoid (histologically 5 BT, 1 BB/BT, and 1 compatible with treated tuberculoid leprosy), and eight as lepromatous (histologically 4 BL, 3 LL, 1 biopsy lost). The lepromatous patients fell into two distinct groups. The group with "active disease" consisted of five patients with no or only erratic treatment, Bacteriologic Indices (BI) between 4.0 and 5.0, Morphologic Indices (MI) between 3.0 and 8.0%, histologic classification mainly LL (3 LL, 1 BL, 1 biopsy lost), and AFB in their nasal discharge. The group with "inactive disease" consisted of three patients who had been on almost regular treatment for more than one year, who had BI's between 2.0 and 3.0, negative MI's, a histologic classification as BL, and no AFB in their nasal discharge. The tuberculoid patients had a BI of 0.2 in two cases and negative BI's in the remaining patients. The MI's were zero and there were no AFB in the nasal discharge. The tuberculoid patients were also divided into two groups: those treated for less than six months (histologically 4 BT, 1 BB/BT) and those treated for more than six months (histologically 2 BT, 1 compatible with treated tuberculoid leprosy). Thus, four groups were formed with decreasing degrees of probable infectivity of the index patients: lepromatous active, lepromatous inactive, tuberculoid short treatment and tuberculoid long treatment.

Index persons without leprosy for the control group. For each index person with leprosy a person without leprosy was selected as the index person for the corresponding control household. On a given day the first person who attended the same clinic as the leprosy patient, and who had the same sex, age (plus or minus five years), and marital status as the patient, was selected, provided there was no leprosy in the household.

Patient contact groups. They consisted of those members of the 15 households with a leprosy patient who had lived in one household with the patient before he had received regular treatment, who had no leprosy themselves, and who had no scars from BCG vaccination. The age and sex distribution of the 90 contacts included in the final analysis of the LTT results are given in Table 1, grouped according to the degree of probable infectiousness of the index patients. The 15 households had a total of 175 members of whom 159 were present at the time of examination. Among those, 135 were considered contacts. Four contacts of lepromatous patients were diagnosed as having leprosy and were exluded from the healthy contact group. The complete examination was performed on 100 contacts. The other persons were not available because they a) were considered too young by their parents (28), b) were too sick (2), or c) refused the blood test (1). Results from eight individuals had to be excluded later because of technical faults in the LTT, and results from three persons were excluded because of BCG scars. According to the duration of exposure to the index patient, the contacts of active lepromatous patients were subdivided into a group with less than three years of exposure and another group with more than three years of exposure. Among the 22 household contacts of tuberculoid patients with short treatment, 14 were unlikely to have had contact with the source of infection of their index patient; while all 15 contacts of tuberculoid patients with long treatment might have had such outside contact.

Control group. This consisted of the members of the 15 control households. The age and sex distribution of the 91 control persons included in the final analysis of the LTT results are given in Table 1. The 15 households had a total of 169 members of whom 155 were present at the time of examination. The complete examination was performed on 110 individuals. The other persons were not available because they a) were considered too young by their parents (40), b) were too sick (3), or c) refused the blood test (2). Results from eight individuals had to be excluded because of

TABLE. 1. *Sex and age distribution of patient contact and control groups.*

		Number of males and females in each group						
		Age in years						
		6-14		15-49		≥ 50		
		M	F	M	F	M	F	Total
Patient contact groups								
Household contacts of lepromatous patients	With "active" disease	3	5	11	9	0	7	35
	With "inactive" disease	5	3	3	6	1	0	18
	Total	8	8	14	15	1	7	53
Household contacts of tuberculoid patients	Treated for < 6 months	3	2	2	8	4	3	22
	Treated for > 6 months	2	1	5	6	0	1	15
	Total	5	3	7	14	4	4	37
Total		13	11	21	29	5	11	90
Control group		11	12	18	34	9	7	91
Total		24	23	39	63	14	14	181

technical faults in the LTT's. The LTT results of 11 persons with BCG scars (age 6 to 19 years) were excluded from the results of the control group but compared in a separate analysis with the results of controls of the same age group with no BCG scars.

Examination of the households. Most members of the households were examined in their home villages. The examinations were all performed by the same physician with the help of the same interpreter. Standard questionnaires were used to record information pertaining to the socio-economic status of the household, namely the number of houses, cattle, and ensete plants, and its members: name, sex, and estimated age. The duration of exposure, i.e., household contact, to the index patient with leprosy was determined through interrogation. As a measure of closeness of exposure it was recorded whether a household contact a) lived in the same house as the index patient, thus sharing meals and sleeping place, b) had the same sex as the index patient, thus sharing additional daytime activities, and c) was married to the patient. As only one of the patients with active lepromatous leprosy was married, spouse status was not included in the analysis of the LTT results. The complete examination included a physical examination to check for leprosy and scars from BCG vaccination, and the taking of samples for the laboratory tests, namely slit skin smears from one earlobe, smears of the nasal discharge and venous blood for the LTT. None of the contacts without leprosy and none of the controls had AFB in their skin smears or smears from nasal discharge. Evidence of past or present tuberculosis existed in two of the leprosy households and in three of the control households. The patient contact and control groups are similar with respect to the number of persons per household (11.5) and per house (4.5), as well as the number of ensete plants (30) and cattle (0.5) per person. The sex and age distribution among groups are also comparable (see Table 1).

Laboratory methods. *Microscopic examination of skin smears for AFB.* Skin smears were processed and scored for BI and MI in a routine fashion.

Microscopic examination of smears from nasal discharge. Subjects were asked to blow their noses after moistening with distilled water if dry, collecting the discharge into plastic bags. The material was then smeared onto slides and processed as a skin smear. The

slides were examined for five minutes under the microscope (oil immersion, 800X) and labeled as positive when AFB were seen and negative when no AFB were seen.

Histological examination of skin biopsies. Biopsies were taken from skin lesions suspected to be due to leprosy and then processed and classified according to Ridley and Jopling ([29]).

Lymphocyte transformation test. A micromethod of the LTT, as described by Closs ([6]), was used with several modifications ([1]). Five to 20 ml of venous blood were drawn, heparinized and transported to the laboratory in glass containers at ambient temperatures of around 22°C. The blood samples were always processed immediately and incubated within 15 hours after drawing. The lymphocytes were separated by centrifugation on Ficoll-Isopaque (Pharmacia, Nyegaard and Co.), washed and cultured in microtiter trays in a final concentration of 0.5×10^7 cells per ml medium TC 199 (Flow Laboratories) supplemented by 10% pooled serum from healthy persons from areas without leprosy. Stimulation with antigens was always done in triplicate and with two or three concentrations of each antigen. Parallel stimulation of the lymphocytes with phytohemagglutinin was performed to check culture conditions and cell viability. Nine cultures on each antigen tray and three cultures on the PHA tray were left unstimulated to serve as control cultures. Even though the analysis revealed differences between the unstimulated cultures in some cases, it could be excluded that they influenced the results, except in three cases which will be reported in the Results section. In some cases, because of a shortage of lymphocytes, duplicate cultures had to be used instead of triplicate cultures, and not all concentrations of the different antigens could be tested. Cultures stimulated with PHA were harvested on day four and antigen-stimulated cultures on day seven. Sixteen hours before harvest 0.5 μCi of tritium-labeled thymidine was added to the cultures. Cells were collected on glass fiber filters, washed with distilled water, dried and counted in a liquid scintillation counter. Thymidine incorporation was measured as counts per minute (cpm).

M. leprae antigens. Leprosy bacilli were obtained from biopsies of 33 lepromatous patients with high BI's and MI's. From this pool, preparations of whole, washed bacilli (*M. leprae* "whole") and of sonicated bacilli (*M. leprae* "son.") were prepared as described by Bjune *et al* ([1]). They were used in final concentrations of 10^6, 10^7, and 10^8 bacilli per ml culture medium.

BCG. Whole BCG bacilli (dried BCG vaccine, Glaxo Laboratories Ltd.) were added to the culture in a final concentration of 10^6 and 10^7 bacilli per ml culture medium.

M. avium and M. gordonae. Strains described in a previous publication ([19]) were taken, and prepared in a similar manner. Whole bacilli were used in final concentrations of 10^7 and 10^8 bacilli per ml culture medium.

PHA. PA grade (Wellcome Research Laboratories) was used in a final concentration of 1:100.

Statistical analysis. *Intra-triplicate variation.* The coefficient of variation (CV) was used to calculate the intra-triplicate variation of thymidine incorporation in the lymphocyte cultures on a 10% random sample. The arithmetic means of the CV's were 19% for the PHA cultures, 32% for the antigen stimulated cultures, and 41% for the unstimulated cultures.

Net LTT responses. The differences between the mean cpm of the triplicate stimulated cultures and the mean cpm of the corresponding unstimulated cultures yielded the "net LTT responses" which were used in the analysis. The net LTT responses were calculated for PHA and all antigen concentrations. Comparisons between the different exposure groups were then made within all levels of concentration. The biggest differences were found where the majority of persons had their peak responses, namely at the following concentrations: 10^7 bacilli per ml culture medium (*M. leprae* "whole" and BCG); 10^8 bacilli per ml culture medium (*M. leprae* "son.," *M. avium* and *M. gordonae*). The following evaluation is based on the net LTT responses for these concentrations unless otherwise indicated. For some purposes individuals were divided according to their net LTT responses into "responders" (net LTT responses 500 or more cpm) and "nonresponders" (net LTT responses below 500 cpm).

Statistical methods used. The net LTT responses were found not to follow a Gaussian distribution. Therefore the data have been analyzed using nonparametric methods,

namely the U-test of Wilcoxon, Mann and Whitney for the comparison of two groups of persons, and the rank correlation coefficient of Spearman for correlations between the responses to different antigens. A p value of 0.05 or less was chosen as an indication of statistical significance.

Analysis of the net LTT responses. The statistical analysis of the data was performed in three different ways:

I. "Matched household analysis." The net LTT responses of all individuals were ranked within each household pair, comprising a household with leprosy and its matched control household. For each household a ratio was then determined of the mean rank of the single household to the sum of the mean ranks of the two households comprising the pair. These "weighted ranks" of patient contact households with the same type of exposure to leprosy were then compared with the weighted ranks of the corresponding control households using the U-test of Wilcoxon, Mann and Whitney.

II. "Partly pooled analysis." All individuals with the same type of exposure to leprosy in the household were pooled as were their matched controls. The net LTT responses were then compared between exposure types, i.e., contacts of lepromatous patients and corresponding controls.

III. "Completely pooled analysis." In contrast to the partly pooled analysis, all persons without exposure to leprosy in the household were pooled in one single control group. Comparisons involving controls were always made with this same pooled control group. In addition, comparisons were made between groups of contacts with exposure to different types of leprosy in the household, e.g., contacts of lepromatous patients and contacts of tuberculoid patients.

The three types of analysis yielded essentially the same results. In order to simplify the

TABLE 2. *Responses in lymphocyte transformation tests (LTT) against* M. leprae *antigen preparations and phytohemagglutinin (PHA) in household contacts of leprosy patients and in controls. The number of individuals with cultures (n), and medians ($\tilde{x}$) and interquartile ranges (Q_3-Q_1) of the net LTT responses[a] are given.*

	Net LTT responses to											
	M. leprae "whole" counts/min.			*M. leprae* "son." counts/min.			Unstim. Cultures counts/min.			PHA counts/min.		
Group	n	$\tilde{x}$	Q_3-Q_1	n	$\tilde{x}$	Q_3-Q_1	n	$\tilde{x}$	Q_3-Q_1	n	$\tilde{x}$	Q_3-Q_1
I Household contacts of												
Active lepromatous patients	35	773	1,263	35	422	1,620	35	1,142	864	29	31,183	54,266
Inactive lepromatous patients	18	158	591	17	57	552	18	490	867	18	72,385	61,153
All lepromatous patients	53	452	1,225	52	350	1,555	53	1,009	966	47	42,346	61,474
Tuberculoid patients treated < 6 months	22	-72	923	19	-75	780	22	1,575	1,608	20	30,387	23,812
Tuberculoid patients treated > 6 months	14	390	1,574	15	56	1,231	15	1,072	1,352	15	34,104	46,791
All tuberculoid patients	36	24	1,044	34	7	662	37	1,314	1,460	35	30,554	29,762
All leprosy patients	89	287	1,148	86	172	1,186	90	1,050	1,130	82	34,520	45,170
II Control group	87	106	658	85	54	588	91	584	958	83	44,328	39,823

[a] Difference between mean counts per minute of stimulated triplicate and mean counts per minute of unstimulated triplicate.

presentation in the paper, we report only results of the "completely pooled analysis," unless otherwise indicated.

RESULTS

LTT responses to antigens of *M. leprae*. *Influence of the type of leprosy in the index person.* The medians and interquartile ranges of the LTT responses to *M. leprae* "whole" and *M. leprae* "son." in household contacts of patients with different types of leprosy and the control group are presented in Table 2. The median LTT responses are low among the controls, slightly higher among the contacts of all leprosy patients, and again higher among the contacts of lepromatous patients. The highest median LTT responses are found among the contacts of active lepromatous patients. Thus, the median LTT responses show generally an increase as the contacts are exposed to patients with increasing degrees of probable infectivity (Fig. 2).

When the differences between the LTT responses to *M. leprae* antigens of the various patient contact groups and the control group are tested for significance, one obtains the results presented in Table 4. While household contacts of all leprosy patients do not respond significantly differently from the controls, household contacts of lepromatous patients do respond significantly more strongly. The differences are even more pronounced when only the contacts of active lepromatous patients are chosen for the comparison with the controls. Contacts of active lepromatous patients and contacts of all lepromatous patients have significantly stronger responses than contacts of tuberculoid patients. The latter are not different from the controls. Within the group of tuberculoid patients, contacts of patients with short treatment have signifi-

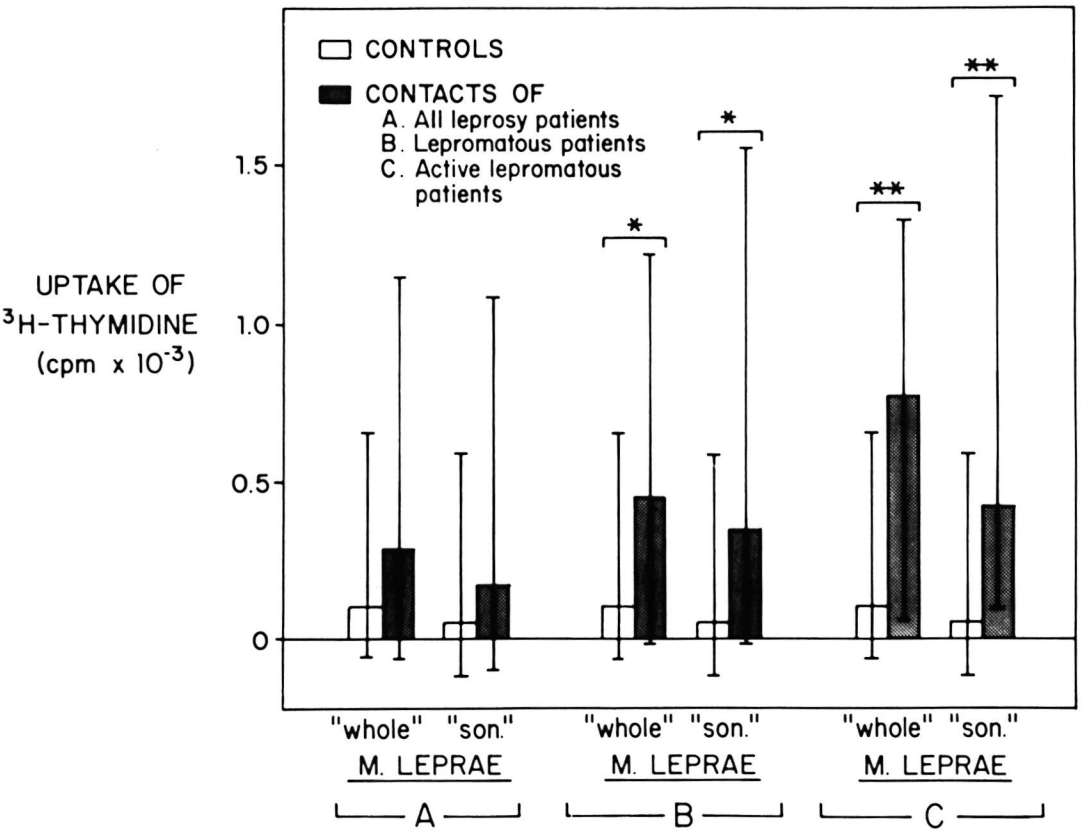

FIG. 2. Median responses and interquartile ranges of lymphocyte transformation tests (LTT)[a] against *M. leprae* antigen preparations in patient contact groups with increasing exposure to leprosy bacilli in the household. Asterisks indicate significant differences (*: $p<0.05$; **: $p<0.01$).

[a]Based on the net LTT responses, i.e., the differences between mean counts per minute of stimulated triplicate and mean counts per minute of unstimulated triplicate.

cantly lower LTT responses than contacts of patients with long treatment and controls. In the latter comparison the data may have been distorted by significant differences in the unstimulated cultures ($p < 0.01$) which had the opposite direction.

A division of the individuals, according to their net LTT responses to *M. leprae* "whole," into responders and nonresponders gives the following percentages of responders: 26% in the control group, 39% among the contacts of leprosy patients, 31% among the contacts of tuberculoid patients, and 57% among the contacts of active lepromatous patients. Even though the contacts of active lepromatous patients have the highest percentage of responders of all tested groups, there are 15 individuals in this group who do not respond well to *M. leprae* "whole" (10^7 bacilli per ml culture medium) despite the exposure to highly bacilliferous patients. Of these 15 contacts who do not respond at this concentration of antigen, eight are also unresponsive to the other concentrations of *M. leprae* "whole" and to all concentrations of *M. leprae* "son." All except one of these contacts also have low responses to PHA, but six responded to the other mycobacteria tested. The eight individuals who did not respond at all to *M. leprae* antigens are not different from the responders with regard to sex, age and state of consanguinity with the index patient ([23]).

Influence of the duration and closeness of exposure. Contacts of active lepromatous patients who had more than three years of exposure to the index patient, who had the same sex as he, and who lived in the same house as he did had higher median LTT responses to *M. leprae* "whole" than contacts who had less than three years of exposure, who had the opposite sex, and who lived in another house of the compound, respectively. None of the differences was statistically significant, however.

Comparison between M. leprae "whole" and M. leprae "son." The median LTT responses to *M. leprae* "whole" are higher than those to *M. leprae* "son." throughout (Table 2 and Fig. 2). Also, differences between the various patient contact groups and the control group are found more often and with statistically higher significances with *M. leprae* "whole" than with *M. leprae* "son." (Table 4). This finding is more pronounced in the "partly pooled analysis." There the comparison between household contacts of active lepromatous patients and their matched controls yields statistical significances of $p < 0.0001$ for *M. leprae* "whole" and of $p < 0.02$ for *M. leprae* "son." The comparison between the contacts of all lepromatous patients and their matched controls shows significant differences between the responses to *M. leprae* "whole" ($p < 0.01$) but not between the responses to *M. leprae* "son." ($p > 0.05$).

LTT responses to PHA. The LTT responses to PHA are shown in Table 2. The following differences were found to be statistically significant (Table 4): household contacts of active lepromatous patients respond significantly less to PHA than contacts of inactive lepromatous patients and controls. These results may have been distorted by opposite trends in the unstimulated cultures ($p < 0.01$). Contacts of inactive lepromatous patients respond significantly more to PHA than the controls. Contacts of tuberculoid patients respond significantly less than the controls. However, this difference is not found in the "matched household analysis" and the "partly pooled analysis." Household contacts of active lepromatous patients with less than three years of exposure to the patient had significantly higher LTT responses to PHA ($p < 0.01$) than contacts with more than three years of exposure. There was no evidence of an influence of the indicators of closeness of exposure on the LTT responses to PHA in contacts of active lepromatous patients.

LTT responses to BCG, *M. avium* and *M. gordonae*. Table 3 shows that the median responses to BCG, *M. avium and M. gordonae* are generally much higher than the median responses to antigens from *M. leprae* which they parallel to some extent (Fig. 3). Still, a comparison of the weighted ranks of the households with the highest responses to all mycobacteria, namely the households with active lepromatous patients, with the weighted ranks of their matched control households shows clearly that the differences between the two groups of households are much greater for *M. leprae* antigens than for the other mycobacteria (Fig. 4). No significant differences were found for any of the mycobacteria other than *M. leprae* (Table 4) with one exception: in the "partly pooled analysis" household contacts of tuberculoid patients respond significantly more to BCG and *M. avium* ($p < 0.05$) than the corresponding controls.

Cross-reactivity of *M. leprae* with other mycobacteria in the LTT. When the LTT responses of all 150 individuals with complete LTT's are tested for a correlation between the responses to the different mycobacteria, a significant correlation ($p < 0.001$) is found between antigens for all the pairs of antigens (Table 5). The Spearman rank correlation coefficients are higher between *M. leprae* "son." and the other mycobacteria than between *M. leprae* "whole" and the other mycobacteria.

Persons with scars from BCG vaccination, and with no exposure to leprosy in the household, were compared with age-matched persons from the control group. The individuals with BCG scars had higher median LTT responses than the persons without BCG scars for PHA and all antigens (Table 6). The differences reached statistical significance for BCG, *M. leprae* "son." and PHA ($p < 0.05$) for all stimulants).

DISCUSSION

In an area endemic for leprosy, healthy household contacts of lepromatous patients had significantly higher cellular immune responses to *M. leprae* antigen than healthy contacts of tuberculoid patients and healthy persons without household contact to leprosy. The degree of sensitization, as indicated by the LTT response, in the difference exposure groups paralleled therefore the degree of probable infectivity of the index patient. Our interpretation is that the sensitization in the household contacts of lepromatous patients is directed specifically against antigens of *M. leprae* because the parallel examination of the cellular immune responses to the other three locally important mycobacteria, namely *M. tuberculosis*, *M. avium*, and *M. gordonae*, did not reveal significant differences between the patient contact and control groups. This is so despite the substantial degree of cross-reactivity we found between the LTT

TABLE 3. *Responses in lymphocyte transformation tests (LTT) against BCG,* M. avium *and* M. gordonae *in household contacts of leprosy patients and in controls. The number of individuals with cultures (n), and medians ($\tilde{x}$) and interquartile ranges (Q_3-Q_1) of the net LTT responses*[a] *are given.*

	Net LTT responses to								
	BCG counts/min.			*M. avium* counts/min.			*M. gordonae* counts/min.		
Group	n	$\tilde{x}$	Q_3-Q_1	n	$\tilde{x}$	Q_3-Q_1	n	$\tilde{x}$	Q_3-Q_1
I Household contacts of									
Active lepromatous patients	35	2,608	7,191	35	853	2,179	35	1,879	3,274
Inactive lepromatous patients	17	676	2,762	15	355	952	14	592	1,532
All lepromatous patients	52	1,530	4,768	50	676	1,859	49	1,069	3,302
Tuberculoid patients treated <6 months	21	565	3,548	20	625	1,884	19	681	1,775
Tuberculoid patients treated >6 months	14	1,576	4,263	14	657	1,635	14	1,380	2,466
All tuberculoid patients	35	1,152	3,420	34	657	1,875	33	1,234	2,006
All leprosy patients	87	1,357	4,185	84	674	1,781	82	1,157	2,656
II Control group	90	532	3,888	80	291	2,673	77	829	3,339

[a]Difference between mean counts per minute of stumulated triplicate and mean counts per minute of unstimulated triplicate.

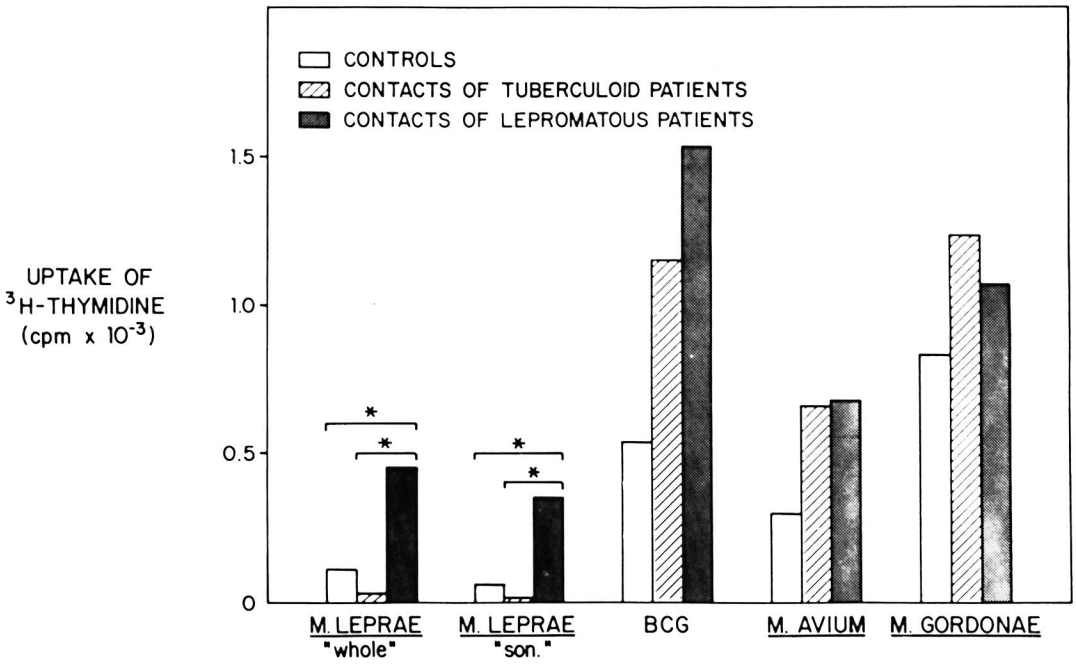

FIG. 3. Median responses in lymphocyte transformation tests (LTT)[a] against *M. leprae* antigen preparations, BCG, *M. avium* and *M. gordonae* in household contacts of tuberculoid patients, household contacts of lepromatous patients and in controls. Asterisks indicate significant differences (*: $p<0.05$).

[a]Based on the net LTT responses, i.e., the differences between mean counts per minute of stimulated triplicate and mean counts per minute of unstimulated triplicate.

responses to the different mycobacteria. Closs ([6]) also demonstrated cross-reactivity between *M. leprae* and *M. tuberculosis* using a similar technic.

The specific cellular immune responses to *M. leprae* antigen in a high proportion of healthy contacts of leprosy patients provide substantial evidence that subclinical infection with *M. leprae* is common. It is likely that a small proportion of the sensitized persons in our study have had undetected self-healing leprosy in the past ([2, 22]). Another few persons may have been in the incubation period of leprosy at the time of the examination. Yet only a small number of those with specific sensitization can be accounted for by past or future clinical disease as the total incidence of clinical leprosy is known to be very small ([10, 17, 20]). Our investigation confirms the basic findings of Godal and co-workers ([13, 15]), and Myrvang ([24]), and extends the observations with respect to influence of the degree of infectivity of the index patient. In the studies of Godal *et al* ([13, 15]), there did not seem to be the spectrum of sensitization related to the degree of infectiousness of the index patient that was demonstrated in our present study. The apparent differences are likely related to the differences in the selection of the patient contact and control groups and possibly also to differences in the sensitivity of the LTT technic.

The further subdivision of lepromatous patients according to their activity demonstrated that it was only the active lepromatous patients that accounted for the significant degree of sensitization to *M. leprae* in the household contacts of lepromatous patients. The active lepromatous patients in our study were highly bacilliferous and shed AFB prolifically in their nasal discharges, which is probably the most important portal of exit ([27, 32]). The duration of exposure to an active lepromatous patient did not seem to influence the degree of sensitization to *M. leprae*. However, contacts who can be considered to have had the closest exposure because they lived in the same house and had the same sex as the index patient had higher median responses than the other contacts.

Perhaps the most important observation is that despite the heavy exposure to leprosy

TABLE 4. *Statistical comparison of the responses in lymphocyte transformation tests (LTT)*[a] *against* M. leprae *antigen preparations, BCG,* M. avium, M. gordonae *and phytohemagglutinin (PHA) in groups of persons with different exposure to* M. leprae *in the household.*

Group I	Group II	p values[b]					
		M. leprae "whole"	*M. leprae* "son."	BCG	*M. avium*	*M. gord.*	PHA
Household contacts of all leprosy patients	Controls	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Household contacts of all lepromatous patients	Household contacts of all tuberculoid patients	<0.05	<0.05	N.S.	N.S.	N.S.	N.S.
	Controls	<0.05	<0.05	N.S.	N.S.	N.S.	N.S.
Household contacts of all tuberculoid contacts	Controls	N.S.	N.S.	N.S.	N.S.	N.S.	<0.05[c]
Household contacts of active lepromatous patients	Household contacts of inactive lepromatous patients	<0.05	N.S.	N.S.	N.S.	N.S.	<0.01[c]
	Household contacts of all tuberculoid patients	<0.01	N.S.	N.S.	N.S.	N.S.	N.S.
	Controls	<0.01	<0.01	N.S.	N.S.	N.S.	<0.05[c]
Household contacts of inactive lepromatous patients	Controls	N.S.	N.S.	N.S.	N.S.	N.S.	<0.05
Household contacts of tuberc. patients treated <6 months	Household contacts of tuberc. patients treated >6 months	<0.05[c]	N.S.	N.S.	N.S.	N.S.	N.S.
	Controls	<0.05[c]	N.S.	N.S.	N.S.	N.S.	N.S.
Household contacts of tuberc. patients treated >6 months	Controls	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

[a] Based on the net LTT responses, i.e., the differences between mean counts per minute of stimulated triplicate and mean counts per minute of unstimulated triplicate.

[b] The p values are based on the application of the U-test of Wilcoxon, Mann and Whitney. P values of <0.05 or <0.01 not marked (c) indicate stronger responses in group I than in group II. N.S.: p values not significant.

[c] The p values marked (c) indicate that the response is stronger in group II.

bacilli, some contacts of the highly bacilliferous patients demonstrated virtually no reactivity to *M. leprae* antigen. It is among these contacts that one would predict future lepromatous patients in accordance with Dharmendra and Chatterjee's findings ([9]) in lepromin-negative contacts of lepromatous patients. This group should be considered at high risk and deserves careful follow-up. Future work may demonstrate that it is these contacts that would benefit from drug prophylaxis and that this would be valuable in reducing the numbers of patients who become disseminators of leprosy prior to diagnosis.

The household contacts of tuberculoid patients in our study appear to be a heterogeneous group with a great variability of their responses to *M. leprae* antigens. Further investigations are required to determine whether the statistical differences found between the two subgroups with different duration of treatment and the controls remain

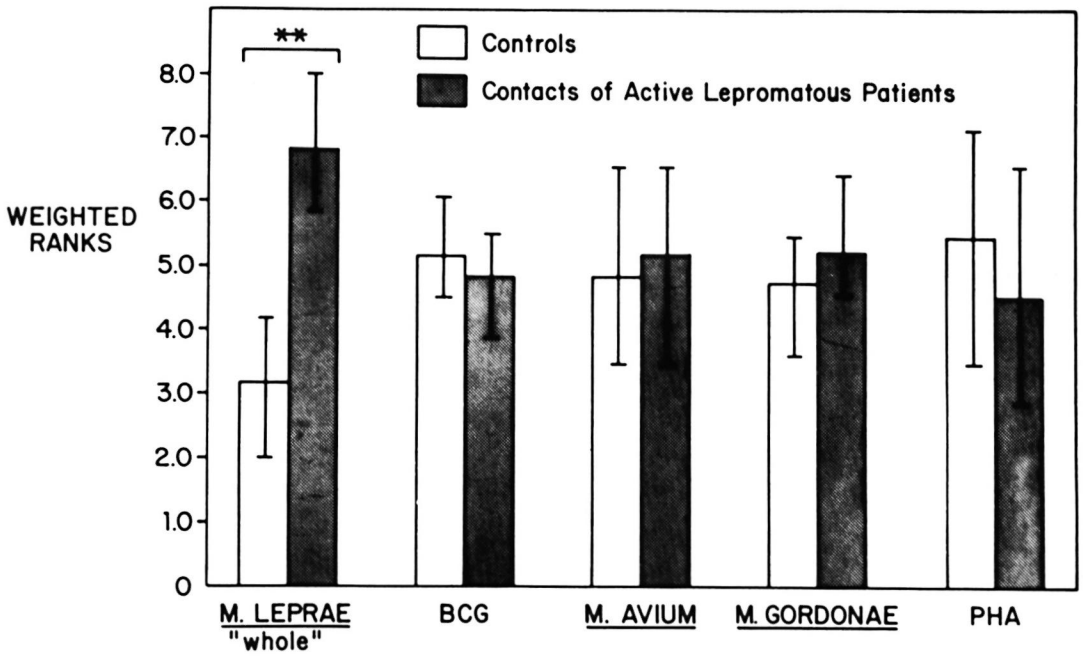

FIG. 4. Responses in lymphocyte transformation tests (LTT)[a] against *M. leprae* "whole," BCG, *M. avium*, *M. gordonae* and phytohemagglutinin (PHA) in households with active lepromatous patients and in matched control households. The means and ranges of the ranks weighted within each household pair are given. Asterisks indicate statistical significance (**: $p < 0.01$).

[a]Based on the net LTT responses, i.e., the differences between mean counts per minute of stimulated triplicate and mean counts per minute of unstimulated triplicate.

TABLE 5. *Correlation between the responses to different mycobacterial antigens in the lymphocyte transformation test (LTT).*[a]

	Spearman rank correlation coefficient[b] r_s			
	M. leprae "son."	BCG	*M. avium*	*M. gordonae*
M. leprae "whole"	0.58	0.39	0.27	0.37
M. leprae "son."		0.49	0.44	0.57
BCG			0.72	0.79
M. avium				0.69

[a]Based on the net LTT responses, i.e., the differences between mean counts per minute of stimulated triplicate and mean counts per minute of unstimulated triplicate, in 150 LTTs.

[b]The Spearman rank correlation coefficient has a p value of < 0.001 in all cases.

significant if the groups are more comparable with regard to the responses of the unstimulated cultures and the likelihood of exposure to the source of infection of their index patients.

The finding that household contacts of active lepromatous patients have significantly lower responses to PHA than contacts of inactive lepromatous patients and controls makes it unlikely that differences in the lymphocyte culture conditions are the cause for the observed differences between the LTT responses to *M. leprae* antigens. Whether or not the LTT responses to PHA are truly suppressed among the contacts of active lepromatous patients cannot be decided on the basis of our data.

The results of our study have shown that for use in the LTT the antigen preparation from whole bacilli is a more sensitive and specific indicator of sensitization to *M. leprae* than the sonicated preparation.

TABLE 6. *Responses in lymphocyte transformation tests (LTT) against* M. leprae *antigen preparations, phytohemagglutinin (PHA), BCG,* M. avium, *and* M. gordonae *of individuals 6-19 years old without exposure to leprosy in the household, according to the presense or absence of scars from BCG vaccination. The numbers of individual cultures (n), and medians ($\tilde{x}$) and interquartile ranges (Q_3-Q_1) of the net LTT responses are given.*

Antigen or mitogen	Scars from BCG vaccination present	n	$\tilde{x}$	Q_3-Q_1
Unstimulated[b]	Yes	11	1,161	991
	No	33	820	976
M. leprae "whole"	Yes	11	803	1,749
	No	31	259	889
M. leprae "son."	Yes	11	770	3,275
	No	31	108	668
PHA	Yes	11	65,952	51,017
	No	29	48,232	39,098
BCG	Yes	11	3,550	12,481
	No	33	501	2,446
M. avium	Yes	11	837	4,730
	No	27	274	2,673
M. gordonae	Yes	11	3,011	4,421
	No	24	634	3,281

[a]Difference between mean counts per minute of stumulated triplicate and mean counts per minute of unstimulated triplicate.

[b]Data refers to actual counts per minute in unstimulated cultures rather than net LTT.

Since a high proportion of close contacts of patients with active lepromatous leprosy develop an increased sensitization to *M. leprae* but do not develop clinical disease, it is of interest to examine such contacts for additional risk factors which might influence the host resistance to *M. leprae*, namely, sex and age of the exposed person and the state of consanguinity with the patient. We will present such an evaluation of our data in another publication ([23]).

SUMMARY

Fifty-three household contacts of lepromatous patients, 37 household contacts of tuberculoid patients, and 91 control persons were examined with the lymphocyte transformation test (LTT) for their responses to whole and sonicated antigen preparations from *M. leprae*, to BCG, *M. avium*, *M. gordonae*, and phytohemagglutinin (PHA). The study was carried out in the Gurage area of Ethiopia in 15 households with a leprosy patient and 15 matched control households.

Household contacts of lepromatous patients showed significantly greater LTT responses to antigens from *M. leprae* than the controls, whereas household contacts of tuberculoid patients did not respond differently from controls. Household contacts of lepromatous patients had significantly greater responses to *M. leprae* antigens when the index patients were "active," i.e., highly bacilliferous, than when they were "inactive," i.e., having a low bacillary load. The degree of sensitization, as indicated by the LTT response, in different exposure groups paralleled the degree of probable infectivity of the index patient.

A preparation of antigen from whole *M. leprae* proved to be more sensitive and more specific in the LTT than did a sonicated preparation. A significant degree of cross-reactivity was found among the various mycobateria in their LTT responses.

RESUMEN

Empleando la prueba de la transformación de linfocitos (PTL), se estudiaron 53 contactos familiares de pacientes con lepra lepromatosa, 37 contactos de pacientes con lepra tuberculoide y 91 personas control (no contactos). Como antígenos se usaron las preparaciones totales y los sonicados de *M. leprae*, BCG, *M. avium* y *M. gordonae*. También se usó la fitohemaglutinina (PHA). El estudio se llevó a cabo en el área Gurage de Etiopía, en 15 familias con al menos un paciente con lepra por familia y en 15 familias control de condiciones socioeconómicas semejantes.

Los contactos familiares de los pacientes lepromatosos mostraron respuestas (PTL) significativamente mayores con los antígenos del *M. leprae*, mientras que los contactos familiares de los pacientes tuberculoides respondieron de manera similar a los controles. Los contactos familiares de los pacientes lepromatosos tuvieron respuestas al *M. leprae* significativamente mayores cuando los pacientes fueron "activos" (altamente bacilíferos) que cuando fueron "inactivos" (con una baja carga bacilar). El grado de sensibilización, según la PTL, en los diferentes grupos expuestos fue paralelo al grado probable de infectividad del paciente involucrado.

La preparación antigénica con el *M. leprae* entero resultó ser más sensible y más específica en la PTL que el sonicado. También se encontró con esta prueba, un importante grado de reactividad cruzada entre las diferentes micobacterias empleadas.

RÉSUMÉ

On a examiné 53 contacts domiciliaires de malades lépromateux, 37 contacts domiciliaires de malades tuberculoïdes, et 91 personnes témoins, au moyen de l'épreuve de transformation lymphocytaire (LTT) afin d'étudier leur réponse à des préparations antigéniques complètes et préparées par traitement aux ultra-sons, obtenues à partir de *M. leprae*, de BCG, de *M. avium*, de *M. gordonae*, et de phytohémagglutinine (PHA). Cette étude a été menée dans la région de Gurage, en Ethiopie, dans quinze foyers qui comprenaient un malade de la lèpre, et dans quinze foyers témoins assortis de façon appropriée.

Les contacts domiciliaires des malades lépromateux présentaient des réponses de transformation lymphocytaire significativement plus marquées aux antigènes de *M. leprae* que ne le faisaient les témoins. Par contre, les contacts domiciliaires de malades tuberculoïdes ne répondaient pas différemment que les témoins. Les contacts domiciliaires de malades lépromateux témoignaient de réponses significativement plus élevées aux antigènes de *M. leprae*, lorsque les malades avec lesquels ils étaient en contact étaient actifs, c'est-à-dire fortement bacillifères, que lorsque ces malades étaient inactifs, c'est-à-dire avec une charge bacillaire faible. Le degré de sensibilisation, révélé par la réponse de transformation lymphocytaire, dans des groupes différents par leur exposition, correspondait au degré probable d'infectivité des malades avec lesquels les sujets étaient en contact.

Une préparation d'antigènes obtenue à partir de *M. leprae* entier s'est révélée plus sensible et plus spécifique pour l'épreuve de la transformation lymphocytaire, que ne le faisait une préparation obtenue par sonication. Un degré significatif de réactivité croisée a été observé entre les diverses mycobactéries, en ce qui concerne leur réponse à l'épreuve de transformation lymphocytaire.

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