Lymphocyte Transformation Test in Healthy Contacts of Patients with Leprosy II. Influence of Consanguinity with the Patient, Sex, and Age[1,2]

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Exposure to *Mycobacterium leprae* is necessary to acquire leprosy, but most people who are exposed to leprosy bacilli do not contract the disease ([7, 14, 16]). Skin test studies ([17, 27]) and especially the more recent studies with *in vitro* methods of cellular immunology ([10, 12, 19, 22]) show that a high proportion of healthy persons with close exposure to leprosy patients have developed a cellular immune response to *M. leprae*, indicating that they have been infected without developing the disease. One can conclude from these findings that, in addition to the exposure to leprosy bacilli, other factors play an important role for the development of clinical disease.

Several epidemiologic studies point to the presence of a hereditary susceptibility to leprosy ([1, 5, 21, 29]). Since patients with lepromatous leprosy have an associated depression of the cellular immune responses to *M. leprae* ([4, 8, 11, 15, 20]), attempts have been made to show a similar defect in consanguineous contacts of lepromatous patients. However, neither skin test studies ([13]) nor a study using the lymphocyte transformation test ([25]) could conclusively demonstrate such a defect.

The finding that there are more males than females among patients with lepromatous leprosy ([2, 3, 7, 18]) raises the question whether sex is a predisposing factor for the development of this type of the disease. The examination of skin test responses to lepromin in healthy contacts of leprosy patients did not reveal significant differences between male and female contacts ([26, 27]).

Age does not seem to play a primary role as a risk factor in the etiology of leprosy. The age-specific incidence of leprosy appears to be determined by the opportunity for exposure ([2, 3, 6]). In close contacts of patients with leprosy, skin test responses to lepromin have been found in young children ([13, 17, 27]).

We have investigated responses in the lymphocyte transformation test (LTT) to antigens of *M. leprae* in household contacts of tuberculoid and lepromatous patients in a leprosy-endemic area of Ethiopia. In an accompanying paper ([19]) we have demonstrated that exposure to active lepromatous leprosy in the household induces specific cellular immune responses to *M. leprae* antigens in a high proportion of the exposed individuals. In this paper we evaluate the influence of genetic factors, sex and age on the sensitization to *M. leprae* in persons exposed to this bacterium in the household.

POPULATION AND METHODS

Detailed descriptions of the study area, study population and study groups are provided in the accompanying paper as are laboratory methods and methods of statistical analysis ([19]).

Patient contact groups. They consisted of those members of the 15 households with a leprosy patient who had been in contact with the patient before he had received regular treatment, who had no leprosy themselves, and who had no scars from BCG vaccination. Ninety-one household contacts of leprosy

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patients were available for the final analysis of the LTT results. Of those, 35 were contacts of active lepromatous patients, 18 were contacts of inactive lepromatous patients, and 37 were contacts of tuberculoid patients.

Table 1 gives the sex and age distribution, duration of exposure to the index patient, and numbers of persons living in the same house and having the same sex as the index patient for the household contacts of active lepromatous patients, grouped according to their state of consanguinity with a lepromatous patient. Among the contacts of active lepromatous patients, more males (6 of 14) than females (4 of 21) lived in the same house as the index patient, and more males (9 of 14) than females (2 of 21) had the same sex as the index patient.

Control group. It consisted of those members of the 15 control households who had no scars from BCG vaccination. Ninety-one control persons were available for the final analysis of the LTT results.

Examination of the households. The members of the households were all examined by the same physician with the help of the same interpreter using standard questionnaires. Ages had to be estimated. The duration of exposure to the index patient with leprosy was determined through interrogation. As a member 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. Relationships of the healthy household contacts to the index patients with leprosy were recorded and the patient contact groups were divided accordingly into non-consanguineous and consanguineous contacts. The latter individuals were further grouped into first-degree relatives, sharing 50% of the genes with the patient; second-degree relatives, sharing 25% of the genes with the patient; and third-degree relatives, sharing 12.5% of the genes with the patient. In one household with an active lepromatous case, a cousin of the index patient had inactive lepromatous leprosy (histologically BL). His daughter was classified as a first-degree relative because she shared 50% of the genes with a lepromatous patient. The complete examination of the households included a physical examination to check for leprosy and scars from BCG vaccination, and the drawing of venous blood for the LTT.

RESULTS

Influence of consanguinity with a leprosy patient on the LTT responses. *Household contacts of active lepromatous patients.* The LTT responses to *M. leprae* "whole" of household contacts of active lepromatous patients according to their state of consanguinity with a lepromatous patient are presented in Figure 1. There are no significant differences among

TABLE 1. *Distribution of sex, age and indicators of the closeness of exposure to an active lepromatous patient in household contacts with different states of consanguinity with a lepromatous patient.*

State of consanguinity with a lepromatous patient	Number of males and females in each group: Age in years 6-14 M	6-14 F	15-49 M	15-49 F	≥ 50 M	≥ 50 F	Total M	Total F	Indicators of closeness of exposure: Average duration of exposure to index patient (months)	No. of persons with same sex as index patient	No. of persons in same house as index patient	Total no. of persons
First-degree relatives	1	2	3	2	0	2	4	6	39	4	4	10
Second and third-degree relatives	0	1	2	0	0	3	2	4	7	2	1	6
Non-consanguineous contacts	2	2	6	7	0	2	8	11	19	6	5	19
Total	3	5	11	9	0	7	14	21	22	12	11	35

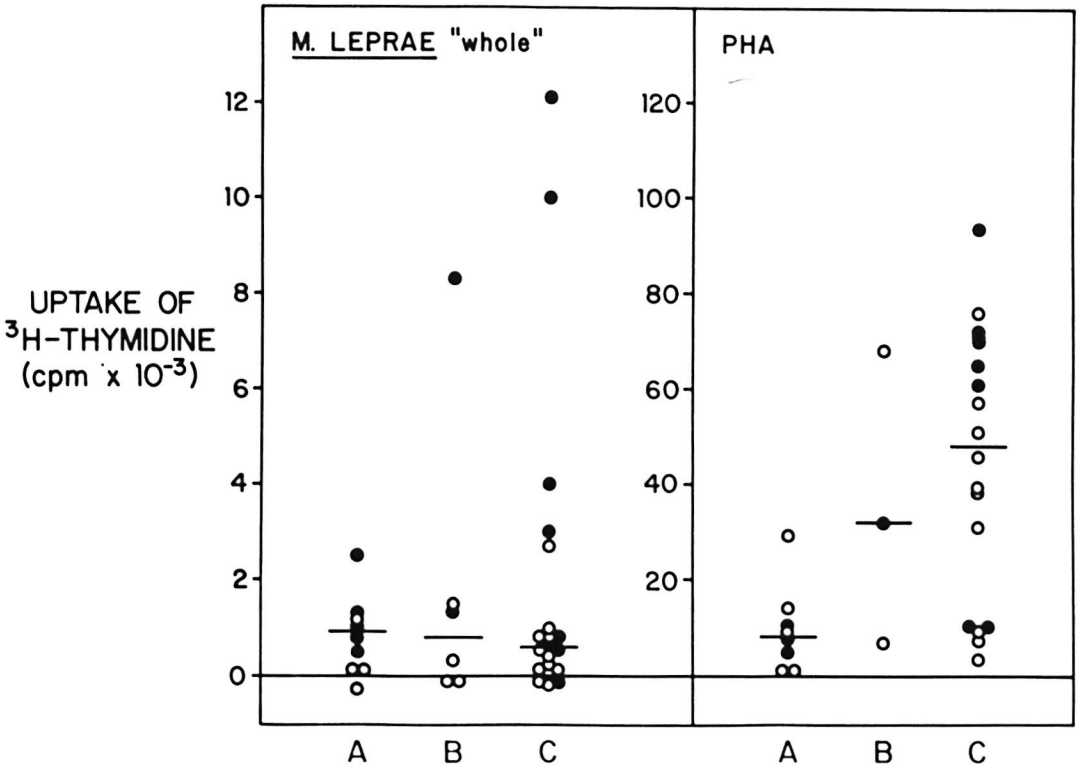

FIG. 1. Responses in lymphocyte transformation tests (LTT)[a] against *M. leprae* "whole" and phytohemagglutinin (PHA) in household contacts of active lepromatous patients with different states of consanguinity with a lepromatous patient. The responses are given for males (●) and females (○); median values are indicated by horizontal lines.

A = First degree relatives
B = Second and third-degree relatives
C = Non-consanguineous contacts

[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.

consanguineous contacts of the first, second and third degree, and non-consanguineous contacts. The median response to *M. leprae* "whole" is even highest among the first-degree consanguineous contacts. There is a higher proportion of responders among the first-degree relatives (7 of 10) than among the second and third-degree relatives (3 of 6), and the non-consanguineous contacts (10 of 19). However, questionable responders are not found among the first-degree relatives of the active lepromatous patients. In contrast, 7 of the 25 less consanguineous contacts show questionable responses.

Responses to PHA (Fig. 1) are significantly lower in consanguineous contacts than in non-consanguineous contacts ($p < 0.05$). Non-consanguineous contacts are not significantly different from the controls. There is no correlation between the responses to *M. leprae* "whole" and to PHA in any of the contact groups.

No significant differences are found between the LTT responses to BCG, *M. avium* or *M. gordonae* among the contact groups with different states of consanguinity with the lepromatous patient.

Household contacts of inactive lepromatous patients and tuberculoid patients. There are no significant differences between the LTT responses to *M. leprae* antigens, any of the other antigens or PHA among the groups with different states of consanguinity with the index patient.

Influence of sex on the LTT responses. *Household contacts of active lepromatous patients.* Males in the households of active lepromatous patients have significantly

stronger responses to *M. leprae* "whole" than females ($p < 0.01$). The median responses of males are higher than the ones of females in all age groups. Out of 14 male household contacts, 12 are responders and 2 are nonresponders; while out of 21 females 8 are responders, 7 are questionable responders, and 6 are nonresponders.

Responses to PHA are not significantly different in males and females. Neither are there any sex differences in the responses to the other mycobacteria tested.

Household contacts of inactive lepromatous patients and tuberculoid patients, and control group. No significant differences exist between males and females in their responses to PHA or any of the antigens tested.

Influence of age on the LTT responses. *Household contacts of active lepromatous patients.* The median LTT responses to *M. leprae* "whole" in the different age groups of contacts of active lepromatous patients are shown in Table 2. Median responses are highest in the youngest age group and lowest in the oldest age group. In the middle age group a further subdivision reveals that contacts who are 15 to 29 years old respond significantly less than contacts who are 30 to 39 years old ($p < 0.02$). The 15 to 29 year old contacts also have lower median responses (142 cpm) than the 6 to 14 year old ones (1,026 cpm) but statistical significance is not reached.

The median responses to PHA (Table 2) decrease with increasing age of the contacts. The Pearson correlation coefficient shows a negative correlation ($p < 0.05$) between PHA responses and age among the contacts of active lepromatous patients.

Lymphocyte responses to BCG (Table 2), *M. avium* and *M. gordonae* are not significantly different in the various age groups of contacts of active lepromatous patients.

Household contacts of inactive lepromatous patients and tuberculoid patients, and control group. In all age groups the median LTT responses to *M. leprae* "whole" are lower among the contacts of inactive lepromatous patients and tuberculoid patients, and among the controls than among the contacts of active lepromatous patients (Table 2). The following differences are statistically significant: contacts of inactive lepromatous patients respond

TABLE 2. *Age-specific responses in lymphocyte transformation tests (LTT) against* M. leprae *"whole," phytohemagglutinin (PHA) and BCG in groups with different household exposure to leprosy. 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.*

Group	Age (years)	*M. leprae* "whole" n	*M. leprae* "whole" $\tilde{x}$ counts/min.	*M. leprae* "whole" Q_3-Q_1	Net LTT responses to PHA n	PHA $\tilde{x}$ counts/min.	PHA Q_3-Q_1	BCG n	BCG $\tilde{x}$ counts/min.	BCG Q_3-Q_1
I Household contacts of Active lepromatous patients	6-14	8	1,026	2,028	8	48,536	53,125	8	2,537	5,352
	15-49	20	800	2,311	16	31,407	54,641	20	3,236	7,629
	≥ 50	7	381	776	5	6,926	22,060	7	1,468	6,743
Inactive lepromatous patients	6-14	8	68	610	8	87,747	59,673	8	287	460
	15-49	9	170	818	9	68,909	54,856	8	2,799	5,278
	≥ 50	1	327		1	21,240		1	1,430	
Tuberculoid patients	6-14	8	177	1,003	7	68,030	39,561	8	530	2,961
	15-49	23	30	1,033	23	27,906	27,555	22	2,014	4,496
	≥ 50	5	-30	4,830	5	23,190	21,062	5	565	1,443
II Control group	6-14	22	230	981	20	57,666	45,388	23	200	1,988
	15-49	52	104	882	51	39,073	43,866	53	679	5,308
	≥ 50	13	105	454	12	34,180	39,854	13	1,044	5,750

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

significantly less than contacts of active lepromatous patients in the age group 6 to 14 years ($p < 0.05$). Contacts of tuberculoid patients and controls have significantly lower responses than contacts of active lepromatous patients in the age group 15 to 49 years ($p < 0.01$ and 0.05, respectively).

The median responses to PHA decrease with increasing age in all patient contact groups and the control group (Table 2).

Responses to BCG, *M. avium* and *M. gordonae* all increase with age in all patient contact groups and the controls with a tendency to decrease again in the old.

DISCUSSION

Household contacts of lepromatous leprosy patients who shared 50% of the genes with the patient did not have lower *in vitro* responses to *M. leprae* antigens than contacts who shared less genetic material, i.e., second and third-degree relatives and non-consanguineous contacts. This applies both to households with active and inactive lepromatous patients. Our finding is in line with the results of Price and his co-workers ([25]), but it does not exclude the possibility that a genetic factor may have an influence on the development of lepromatous leprosy as has been suggested by Newell ([23]). As the incidence of lepromatous leprosy is very low even in close contacts of lepromatous patients ([7, 14, 16]), the few of their consanguineous contacts with negative lymphocyte transformation responses resulting from a hereditary defect would probably not suffice to influence the LTT results of the group of first-degree relatives as a whole. We did find though that three out of ten heavily exposed first-degree relatives of active lepromatous patients were completely unresponsive in the LTT. This could represent a group with a genetically determined lack of responsiveness to antigens of *M. leprae*. A follow-up of all eight non-responders to *M. leprae* antigens among the household contacts of active lepromatous patients might reveal that it is the three first-degree relatives among them who will develop lepromatous leprosy.

It would have been of interest to have information about the HLA[4] types of our consanguineous contacts of active lepromatous patients. De Vries ([29]) had suggested on the basis of his findings in borderline tuberculoid patients that the genetic predisposition to leprosy is linked to the HLA system. His attempts, though, to extend his findings did not reveal the same link in lepromatous leprosy ([30]). Also, Stoner and Tow ([28]), testing LTT responses to *M. leprae* in HLA identical siblings of lepromatous leprosy patients, found no depression of the responses as compared to HLA-nonidentical siblings.

On the basis of our data we cannot decide whether a genetic predisposition to leprosy exists and whether, if it does exist, this predisposition is associated with a lack of responsiveness *in vitro* to *M. leprae* antigens at the first exposure to the antigen.

Male household contacts of active lepromatous patients had significantly higher responses to antigens of *M. leprae* than female contacts. One might rather have expected males to be less responsive in the LTT than females because males account for about two-thirds of the lepromatous patients in our study area as well as in other study areas ([3, 7, 18]), and patients with lepromatous leprosy are known to have low responses to *M. leprae* antigens in the LTT ([4, 11, 15]). Or, considering the low incidence of lepromatous leprosy, one might have expected no differences at all between the LTT responses of males and females. However, our finding may not represent a true difference in the capacity of males and females to be sensitized. It could be secondary to differences in the closeness of contact to the index patient, a thought that has been brought up by Bechelli *et al* ([2]). In our study a higher proportion of males than females had the same sex as the index patient and lived in the same house.

Our study does not provide any evidence for an impaired capacity of males to become sensitized to *M. leprae*, which is in accordance with the results from skin test studies ([26, 27]). Yet, on the basis of our results, one cannot exclude the possibility that the higher responsiveness to *M. leprae* antigens of males, as compared to females, is primarily related to their sex.

Exposure to active lepromatous leprosy in the household induced specific cellular immune responses to *M. leprae* antigens in a number of persons in each of the age groups examined. The high proportion of responders found already in the youngest age group

[4]HLA antigens are the determinants of the immune system's ability to identify "self" and "non-self."

reflects the early exposure to leprosy when contact to a highly bacilliferous patient exists in the household. At the same time it provides evidence that a good sensitizing capacity for *M. leprae* is common in this age. Skin test studies have demonstrated that sensitization to lepromin occurs even at earlier ages than we have tested ([17, 27]). The occurrence in our study of a second peak of responses between age 30 and 49 years may be explained by the fact that 6 of 11 contacts in this age group had their first household exposure to the lepromatous index case only two years prior to the time of examination. These persons may well have exhibited primary responses to leprosy bacilli.

The results of our study are compatible with the view that opportunity for exposure rather than age in itself determines the age-specific findings.

The LTT responses which we obtained to the other mycobacteria reflect the increasing exposure with increasing age, while a decrease of the responses to PHA with increasing age is well known in normal persons ([9, 24]), where it is interpreted as a decline of activity of the cellular immune response.

We were not able to identify conclusively those characteristics of the host which are associated with an impaired capacity to become sensitized to *M. leprae*, a finding traditionally considered to mark the group at risk to develop lepromatous leprosy. Nevertheless, the present study has provided further evidence for the necessity to direct measures for the control of leprosy to young children living together with active lepromatous leprosy patients because the majority of them could be shown to develop positive LTT responses to *M. leprae* indicating that they had been infected with the bacilli.

SUMMARY

The study was carried out in the Gurage area of Ethiopia, where 53 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 preparation from *M. leprae* to BCG, *M. avium, M. gordonae* and phytohemagglutinin. The potential influence of host factors, namely the state of consanguinity with the leprosy patient, sex and age on the LTT responses was evaluated.

In the 35 household contacts of "active," i.e., highly bacilliferous, lepromatous patients, consanguinity with a lepromatous patient was not associated with a significant depression of the LTT responses to *M. leprae* antigens. Male household contacts of active lepromatous patients showed significantly greater LTT responses to *M. leprae* antigens than female household contacts. Possible confounding factors for this finding are discussed. Sensitization of *M. leprae* antigens was present already in a high proportion of the 6 to 14 year old household contacts of active lepromatous patients, which was the youngest age group examined in our study.

No significant results were found in any of the other patient contact groups with regard to the host factors examined.

RESUMEN

En un estudio llevado a cabo en el área Gurage de Etiopia, se valoró la influencia potencial de diversos factores del huésped, principalmente el grado de consanguineidad con el paciente, el sexo y la edad, sobre la prueba de la transformación de linfocitos (PTL) inducida con FHA y con las preparaciones antigénicas integrales y sonicadas de *M. leprae*, BCG, *M. avium* y *M. gordonae*. Se estudiaron las respuestas de 53 contactos familiares de pacientes lepromatosos, 37 contactos familiares de pacientes tuberculoides y de 91 personas normales.

En los 35 contactos familiares de los pacientes "activos" (altamente bacilíferos), la consanguineidad con un paciente lepromatoso no estuvo asociada a una depresión significativa en la respuesta hacia los antigenos del *M. leprae*. Los contactos familiares masculinos de los pacientes lepromatosos activos mostraron respuestas en la PTL significativamente mayores hacia el *M. leprae* que los contactos familiares femeninos. Se discuten los posibles factores responsables de este hallazgo. Una alta proporción de los contactos familiares entre 6 y 14 años de edad de los pacientes lepromatosos activos mostraron ya sensibilización hacia los antigenos de *M. leprae*. Este fue el grupo de edad más joven examinado en el estudio. No se encontraron resultados significativamente diferentes en ninguno de los otros grupos de contactos en relación a los factores del huésped examinados.

RÉSUMÉ

On a utilisé l'épreuve de transformation lymphocytaire pour étudier, dans la région de Gurage, en Ethiopie, 53 contacts domiciliaires de malades lépromateux, 37 contacts domiciliaires de malades

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tuberculoïdes, et 91 personnes témoins. Le but de cette étude était d'étudier la réponse de ces individus à des préparations antigéniques complètes et obtenues par traitement aux ultra-sons, à partir de *M. leprae*, de BCG, de *M. avium*, de *M. gordonae*, et de phytohémagglutinine. L'influence éventuelle des facteurs de l'hôte, en particulier la consanguinité avec un malade de la lèpre, le sexe et l'âge, sur la réponse de transformation lymphocytaire, a été évaluée.

Chez les 35 contacts domiciliaires de cas actifs, c'est-à-dire de malades lépromateux fortement bacillifères, la consanguinité avec un patient lépromateux n'était pas associée avec une diminution significative de la réponse de l'épreuve de transformation lymphocytaire à l'égard des antigènes de *M. leprae*. Les contacts domiciliaires de malades lépromateux actifs, de sexe masculin, ont présenté une réponse à l'épreuve de transformation lymphocytaire significativement plus élevée à l'égard des antigènes de *M. leprae*, que ne le faisaient les contacts domiciliaires de sexe féminin. On discute cependant certains facteurs qui pourraient rendre ces observations ambiguës. Dans un pourcentage élevé des contacts domiciliaires de malades lépromateux actifs, et âgés de 6 á 14 ans, on a déjà observé une sensibilisation aux antigènes de *M. leprae*; ce groupe d'âge est le plus jeune étudié dans cette étude.

Aucun résultat significatif n'a été observé parmi les contacts de malades autres, en ce qui concerne les facteurs de l'hôte qui ont été étudiés.

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