PAPERS AND ORIGINALS

Subclinical Infection in Leprosy

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Summary

Lymphocyte transformation has been used to study the immune response to Mycobacterium leprae among contacts and non-contacts of leprosy patients. Of 26 subjects living in a leprosy endemic area for less than two months none responded to M. leprae; 24% of subjects who had lived in an endemic area for more than a year gave a positive response to M. leprae; more than 50% of individuals with occupational contact of leprosy for more than a year responded; and about 50% of contacts of tuberculoid and treated lepromatous patients responded to M. leprae, while only 22% (4/18) of contacts of lepromatous patients treated for less than six months responded.

It seems that leprosy is more highly infectious than is indicated by the prevalence of the disease and that a subclinical infection commonly follows exposure to M. leprae. The relatively low response found in contacts of active lepromatous patients suggests that in these contacts "superexposure" to M. leprae can bring about a decrease in host resistance.

Introduction

The risk of becoming infected with *Mycobacterium leprae* has remained a controversial issue. A view still prevalent is that subjects become infected only after close and prolonged contact with an infectious patient. This view is based on the observation that there tends to be a family aggregation of leprosy in endemic areas, particularly in families with lepromatous cases. The careful epidemiological investigations of Doull *et al.* (1942) in the presulphone era showed that the risk of acquiring leprosy to a household contact of a lepromatous patient was eight times that of a member of a household not infected with leprosy and four times that of a household contact of tuberculoid leprosy. Moreover, it has been claimed that the risk of acquiring leprosy increases with the duration of contact with a leprosy patient (Badger, 1964).

However, other epidemiological observations are not easily compatible with this view. The explosive spread of leprosy when introduced into a non-endemic area (Wade and Ledowsky, 1952; Leiker, 1960) and the high proportion of patients in many countries who do not know their source of infection (Badger, 1964) suggest that close contact is not always required for the transfer of *M. leprae* from infectious patients to healthy individuals.

The development of the lymphocyte transformation test (Bullock and Fasal, 1971; Godal *et al.*, 1971) and the leucocyte migration inhibition test (Godal *et al.*, 1972 a) have made it possible to measure the immune response to *M. leprae* itself. The lymphocyte transformation test is highly specific for *M. leprae*, the degree of cross-reactivity with *M. tuberculosis* being of the order of 15% or less (Godal *et al.*, 1972 b). Therefore, by this method direct information may be obtained concerning the number of people who develop subclinical infection.

In a preliminary study Godal *et al.* (1972 b) found that most medical attendants working with leprosy patients and household contacts of leprosy patients responded to *M. leprae*. In this report a more detailed presentation of an enlarged study is made.

Subjects and Methods

All together 277 subjects were included in this study, of whom 118 were medical attendants who had been working with leprosy patients for more than a year in various parts of the world. Those from outside Ethiopia came to the All-Africa Leprosy and Rehabilitation Training Centre (A.L.E.R.T.) to attend various training courses. Seventy-one subjects had not been working with leprosy patients, 26 of them having lived in an endemic area for less than two months.

Eighty-eight household contacts were examined. A household contact was defined as a person living in the same compound as a leprosy patient. Most (>80%) lived in the same straw hut and were therefore room contacts of the patient. They were family members who accompanied the patients to a leprosy clinic in Addis Ababa or to clinics in the Debre Berhan and Fiche area

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north of Addis Ababa. The diagnosis of the patients was based on clinical examination and classification made according to the Ridley-Jopling scale (Ridley and Jopling, 1966), in some cases assisted by histopathological and bacteriological (bacterial indices) examinations.

The contacts were allocated to two groups according to the diagnosis of the patient—that is, contacts of tuberculoid patients represented contacts of patients with polar tuberculoid, borderline tuberculoid, or indeterminate leprosy, while contacts of lepromatous patients represented contacts of borderline lepromatous or lepromatous patients. Patients belonging to the first group are regularly negative by bacteriological examination, while in the last group large numbers of bacteria may be found in skin smears and nasal secretions (Shepard, 1962; R. J. W. Rees, personal communication, 1972). The nasal excretion is eliminated during the first months of chemotherapy (Shepard, 1962).

In each case the contacts or the patient or all of them were asked about other cases of leprosy in the household. Contacts in households with known cases of both tuberculoid and lepromatous leprosy were classified as contacts of lepromatous patients. Contacts in households with a second case of unknown type were excluded from the study.

The method of lymphocyte transformation has been fully described elsewhere (Godal *et al.*, 1971). The presence of transformation was assessed after vital staining with acridine orange. It has in our hands been found to be closely correlated to the results obtained with uptake of radioactive thymidine (Godal *et al.*, 1971, 1972 a; Myrvang *et al.*, 1973). In the field 10-ml samples of blood were collected and defibrinated in universal containers with glass beads and 10 ml of medium 199. The bottles were kept at 0°-+4°C until the lymphocyte transformation test was set up; this was never more than 24 hours after the collection of the blood.

In addition to *M. leprae* the response to bacille Calmette Guérin (B.C.G.) was regularly examined. A response of less than 2% to *M. leprae* or B.C.G. was regarded as negative. Moreover, when the response to *M. leprae* was less than 15% of that observed against B.C.G. the test subject was regarded as a non-responder to *M. leprae* irrespective of the strength of response to *M. leprae* itself.

Statistical Analysis.—The significance of the difference in proportions of responders was measured by the χ^2 test, using graphs prepared by Miss M. V. Mussett (Statistical Services Section, National Institute for Medical Research) and based on Mainland's tables (Mainland *et al.*, 1956).

Results

OVERALL FIGURES

None of the 26 subjects who had lived in a leprosy endemic area for less than two months gave a positive response to *M. leprae*. Out of 45 subjects who had been living in an endemic area for at least a year 11 (24%) responded to *M. leprae*. This difference was significant at a 5% level ($P<0.05$). Among 118 subjects with occupational contact with leprosy patients 62 (53%) gave a positive response. This was found to be significantly different from both the two other groups ($P<0.01$). Out of 88 household contacts 39 (44%) responded to *M. leprae*. Though the mean responses to B.C.G. were higher than to *M. leprae* in all groups, about 30% of the subjects with occupational or household contact responded more strongly to *M. leprae* than to B.C.G.

ETHIOPIAN PERSONNEL WORKING AT A.L.E.R.T.

Ethiopian personnel working at A.L.E.R.T. were sub-grouped according to place of work. As shown in table I only 1 out of 12 people not directly working with patients (office personnel) responded to *M. leprae*, while 16 out of 27 (59%) subjects working in hospital wards, etc., responded to *M. leprae*. Out of 17 personnel working in the outpatient department 15 (88%) gave a positive response to *M. leprae*. These subjects were present in rooms where a large number of patients were carefully examined daily. In contrast, no significant difference in the proportion of responders to B.C.G. was found among these groups (table I).

TABLE I—*Lymphocyte Transformation to M. leprae and B.C.G. in Ethiopian Staff Members at the Leprosy Hospital in Addis Ababa*

Place of Work	Response to:	
	M. leprae (No. of responders/ No. studied)	B.C.G. (No. of responders/ No. studied)
Offices (group 1)	1/12 (8%)	10/11 (91%)
Hospital wards, physiotherapy, and shoe workshop (group 2)	16/27 (59%)	24/27 (89%)
Outpatient department (group 3)	15/17 (88%)	16/17 (94%)
Significance	Group 1-2 $P<0.01$; 1-3 $P<0.01$; 2-3 $P>0.05$	No significant difference between groups ($P>0.05$)

HOUSEHOLD CONTACTS

As shown in table II, 14 out of 29 (48%) household contacts of tuberculoid patients were found to respond to *M. leprae*. A similar proportion of responders (24 out of 59, 41%) were found among contacts of lepromatous patients. The response among lepromatous contacts appeared to be related to the treatment status of the patient as 4 out of 18 (22%) of contacts of patients on less than six months of treatment responded while about 50% (20 out of 41) of contacts of patients under treatment for six months or more gave a positive response to *M. leprae*. No similar influence of treatment was observed in contacts of tuberculoid patients. Spouses appeared to respond similarly to genetically related contacts (table II).

TABLE II—*Lymphocyte Transformation to M. leprae and B.C.G. in Household Contacts of Leprosy Patients*

	Response to:	
	M. leprae (No. of responders/ No. studied)	B.C.G. (No. of responders/ No. studied)
Contacts of tuberculoid (T.T., B.T.) and indeterminate patients:		
Total	14/29 (48%)	22/29 (76%)
Contacts of patients treated for <6 months	11/21 (52%)	15/21 (71%)
Contacts of patients treated for ≥6 months	3/8 (38%)	7/8 (88%)
Spouses	3/7 (43%)	5/7 (71%)
Contacts of lepromatous (B.L.-L.L.) patients:		
Total	24/59 (41%)	42/57 (74%)
Contacts of patients treated for <6 months	4/18 (22%)	12/18 (67%)
Contacts of patients treated for ≥6 months	20/41 (49%)	31/38 (82%)
Spouses	4/13 (31%)	8/10 (80%)
Significance	No significant difference between groups ($P>0.05$)	No significant difference between groups ($P>0.05$)

T.T. = Polar tuberculoid. B.T. = Borderline tuberculoid. B.L. = Borderline lepromatous. L.L. = Lepromatous.

Discussion

Our studies indicate that about 50% of subjects with household or occupational contact with leprosy for at least a year have immunological evidence of exposure to *M. leprae*. These findings support the view that the risk of becoming infected with *M. leprae* in leprosy is much higher than is indicated by the prevalence of the disease, which in Ethiopia is of the order of 1%. Moreover, the most common outcome of the interaction between *M. leprae* and the human body appears to be a subclinical infection. The studies on medical personnel indicate that prolonged intimate contact with leprosy patients is not required for transmission, but our studies do not allow any

definite conclusions concerning the precise mode of transmission in leprosy.

Since at least 50% of household contacts of tuberculoid patients show evidence of exposure to *M. leprae*, the much higher incidence of leprosy among household contacts of lepromatous patients (Doull *et al.*, 1942) cannot be explained as due to a higher risk of exposure alone. These studies have, however, shown that there is a lower proportion of responders among contacts of active lepromatous cases as compared to contacts of tuberculoid patients. These non-immune contacts are presumably more at risk of developing clinical leprosy.

This observation was unexpected since the lepromatous patients obviously are the most infectious. Though it was not found to be statistically significant, one may speculate on possible explanations. We did not find any evidence that patients with lepromatous leprosy were more segregated than others. Moreover, the (genetically unrelated) spouses who shared the bed of the patient showed similar conversion rates to other (genetically related) family members. Thus neither segregation nor genetic factors can explain the poor response among lepromatous contacts.

Further studies are needed before the mechanism behind this poor response can be fully characterized. But the tendency of lepromatous contacts to recover immunologically when the patient was put on antileprosy chemotherapy would indicate that their response was suppressed, possibly due to intensive exposure. This may be due merely to the entry of large numbers of bacilli into their body or they may be exposed through a "tolerogenic" route such as the gastrointestinal tract, which has been found to lead to unresponsiveness under experimental conditions (Battisto and Chase, 1963, 1965).

Thus, our observations suggest that the increased risk of acquiring leprosy among contacts of lepromatous patients may be related to a decrease of host resistance caused by "superexposure" to *M. leprae*. To our knowledge, in no other infectious disease has suppression of immunity of this type been reported. But it may be significant that overcrowded housing conditions which would predispose to "superinfection" have been recognized as a risk factor in both leprosy (Doull, 1962) and tuberculosis.

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Diagnosis of Gilbert's Syndrome: Role of Reduced Caloric Intake Test

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Summary

Reduction in caloric intake to 400 a day for 72 hours resulted in a significant increase in the plasma bilirubin concentration in patients with Gilbert's syndrome and in normal subjects. This was due to an increase in unconjugated pigment. There was no significant increase in the bilirubin concentration in patients with liver disease or haemolytic anaemia.

The increase in unconjugated bilirubin was signficantly greater in Gilbert's syndrome than in normals but only when the initial bilirubin concentration was raised. It was usually seen within 24 hours of reducing the caloric intake. An increase of 100% or more suggests that unconjugated hyperbilirubinaemia is due to Gilbert's syndrome. In the normal subjects the unconjugated bilirubin level did not exceed 1·0 mg/100 ml.

The increase in unconjugated bilirubin concentration on reducing the caloric intake may be due to decreased hepatic bilirubin uridine diphosphate glucuronyl transferase activity, which was shown to be present in seven rats starved for 72 hours. The effect of a 400 calorie diet for 24 hours on the unconjugated bilirubin level may distinguish Gilbert's syndrome from other causes of unconjugated hyperbilirubinaemia.

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Introduction

Gilbert's syndrome, also known as idiopathic unconjugated hyperbilirubinaemia or constitutional hepatic dysfunction, is a common cause of unconjugated hyperbilirubinaemia (Gilbert and Lereboullet, 1901). In this disorder there is defective hepatic clearance of bilirubin (Billing *et al.*, 1964; Berk *et al.*, 1970) and also decreased activity of the enzyme bilirubin uridine diphosphate glucuronyl transferase which conjugates bilirubin (Arias and London, 1957; Black and Billing, 1969).

The syndrome is often diagnosed by finding a raised serum level of unconjugated bilirubin in the presence of a normal serum level of conjugated bilirubin and in the absence of any other obvious cause. Diagnosis in this negative manner is clearly unsatisfactory, but additional procedures such as needle biopsy to show histologically normal liver or isotopic bilirubin kinetic studies (Berk *et al.*, 1970) are not usually practical in the investigation of such a common benign condition.

Gilbert and Herscher (1906) observed that the serum bilirubin concentration was higher when subjects were fasting. Felschen