Serological Reactivity and Early Detection of Leprosy Among Contacts of Lepromatous Patients in Cebu, The Philippines

TO THE EDITOR:

One of the challenges in the epidemiology of leprosy is the quest for a tool to indicate the presence of infection prior to the onset of clinically recognized disease. We and others familiar with the long and somewhat indefinite incubation period have been looking for a marker which would allow us

to monitor this preclinical state. The discovery of the phenolic glycolipid-I (PGL-I) antigen of *Mycobacterium leprae* and the subsequent production of its semi-synthetic neoglycoconjugate analogs, by Brennan, Fujiwara, Gigg, *et al.*, have provided at least one such tool ([1, 2, 6–8]). Application of these semi-synthetic antigens now provides us with an instrument and completes a model system to begin the serological dissection of the incubation period in leprosy.

Previously in retrospective studies conducted in Micronesia, we have reported the detection of antibody to whole *M. leprae*, PGL-I, and crossreactive autoclaved *M. smegmatis* up to 2 years prior to onset of clinical disease ([3–5]). In this paper, we present and discuss our preliminary findings from a continuing prospective study on early detection of leprosy, which was initiated in August 1984 in Cebu, The Philippines. The data presented are based upon detection and prevalence of antibody to the semi-synthetic disaccharide (ND-O-BSA) among contacts of new lepromatous patients and a noncontact control population. This synthetic antigen represents a construct of the terminal and penultimate sugars of the PGL-I molecule of *M. leprae*. The contacts are represented by 321 individuals who are household members of lepromatous patients and who have lived in association with the patient(s) for at least 3 years prior to the diagnosis of the index case and start of multidrug therapy. The controls were individuals who were not known to be contacts of lepromatous patients and who have been screened at the Leonard Wood Memorial Skin Clinic and found to be free of leprosy. We found that serum samples from 36 of 321 contacts contained antibodies which reacted with this synthetic antigen. The prevalence of antibody-positive people in the control group from this endemic area was 7 of 401 by ELISA. Thus, the sero-positive rate was 11.2% for contacts and 1.7% for the control group.

In addition to measuring the prevalence of antibody in contacts, we have been following antibody levels in these subjects in a prospective fashion to ascertain the relationship between sero-positivity and development of the disease. Although the length of the incubation period for leprosy is known to be 3 to 7 years or more, we have been able to acquire some preliminary data on household contacts. Our study has been in progress for 2 years, and of the 36 sero-positive contacts we have been following, three have developed leprosy, which represents an 8.3% attack rate for sero-positive contacts. One of the 285 sero-negative contacts has also developed the disease, representing a 0.4% attack rate for sero-negative contacts resulting in an approximately 20-fold higher risk for developing the disease among sero-positive contacts over this 2-year period of observation. It should be noted that these data are preliminary, and higher numbers and a longer observation time will be required to obtain statistical significance of the relative risk of developing leprosy. The ELISA data for the sero-positive contacts who have developed the disease can be seen in Table 1: Case C-177 was antibody-positive 4 months prior to clinical onset; case C-089 was positive for 6 months before clinical onset; case C-013 was sero-positive for more than 18 months before recognition of the disease. An individual serum was considered positive if the reactivity to ND-O-BSA antigen exceeded 0.15 units (OD_{492}). The age at onset was from 13 to 16 years old. The ELISA reactivity found in a subset of 88 of the normal or noncontact controls with an age range of

TABLE 1. *ELISA data for contacts of lepromatous cases developing leprosy.*[a]

Contacts	Specimen no.[b]				
	1	2	3	4	5
ELISA-positive					
C-013	0.48[c]	0.67	0.78	0.54[d]	0.71
C-089	0.07	0.15	0.16	0.24[d]	
C-177	0.05	0.12	0.25	[d]	
ELISA-negative					
C-120	0.01	0.01	0.06[d]	0.03	

[a] Test controls: mean OD_{492} values of pooled sera: negative 0.03, high positive 1.25, low positive 0.34, conjugate 0.02.

[b] Specimen number represents 4- to 6-month time interval between sera collections.

[c] Underlined values indicate positive ELISA test. ELISA is positive when the OD_{492} values are greater than 0.15.

[d] Onset by clinical diagnosis.

TABLE 2. *Classification of the type of early leprosy developed among ELISA-positive and ELISA-negative household contacts of lepromatous patients.*

New cases among contacts	Clinician		Pathologist	
	Leprosy type	BI[a]	Leprosy type	BI[b]
ELISA-positive				
Male—13 yrs (C-013)	I[c]	2.3+	I/BL[d]	4.0+
Male—15 yrs (C-089)	I	1.3+	BL	4.0+
Male—16 yrs (C-177)	I/BL	4.7+	I/BL	5.0+
ELISA-negative				
Male—13 yrs (C-120)	I/TT[e]	0.3+	I	2 AFB

[a] Bacterial index (BI) determined by slit-skin smear.
[b] BI determined from biopsy.
[c] I = indeterminate.
[d] BL = borderline lepromatous.
[e] TT = tuberculoid.

11 to 20 years was 0.03 ± 0.03 S.D. (OD_{492}), and did not differ from the reactivity found in the total control population. In addition, the three sero-positive contacts were found by biopsy to have a bacterial index (BI) of 4 or higher at the time of diagnosis, whereas the sero-negative contact C-120, who developed leprosy, had a BI of 1 (two acid-fast bacilli found). As can be seen in Table 2, the four cases were clinically classified as indeterminate (I). However, when the biopsies were examined by histopathology, the sero-positive cases were classified as borderline lepromatous and the sero-negative case as indeterminate (9). None of the individuals involved in performing the ELISA, reading the biopsy results, or making the clinical evaluation was aware of the others' findings. These findings are consistent with early detection of leprosy.

Another observation of this study was the finding that only 18% of the 106 index cases were associated with the sero-positive contacts. This preliminary data may indicate that about 1 out of 5 lepromatous cases has the ability to successfully spread the organism to other individuals. Thirty-five percent of the families with the sero-positive contacts contained more than one positive individual. This indicated a clustering of sero-reactive individuals around some index cases who may have an increased ability to shed the organism. Another explanation for this phenomenon might be a higher familial susceptibility.

In conclusion, we have found elevated antibody to synthetic ND-O-BSA antigen representing the PGL-I of *M. leprae* in 11.2% of the contacts of multibacillary patients. The presence of these antibodies indicates a possible increased risk of developing leprosy as compared to the normal population and the sero-negative contacts of multibacillary cases. Also, we found that sero-reactivity, which frequently occurred in clusters, was associated with a minority (18%) of the multibacillary index cases.

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