Acknowledgment

Potential conflicts of interest. All authors: no conflicts.

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Clinical Infectious Diseases 2010; 50:936–937

1058-4838/2010/5006-0025$15.00
DOI: 10.1086/650739

Is Phenolic Glycolipid–I Really a Specific Antigen for Leprosy?

Phenolic glycolipid–I (PGL-I) of *Mycobacterium leprae* is a specific antigen for leprosy, and its terminal residue (ie, 3,6-di-*O*-methyl glucose) has not been found in any other natural molecule [1]. Thus, PGL-I is being employed as a diagnostic tool for the detection of early leprosy.

As found in our earlier studies [2, 3], the serum samples obtained from patients with active leprosy cross-reacted with the antigens derived from *Leishmania donovani* (Figure 1*A*), and we also observed the reactivity to leishmanial antigens of the serum samples obtained from patients who tested negative for leprosy bacteriologically [2]. Because PGL-I continues to persist long after a patient has tested negative for leprosy bacteriologically, it is likely that the anti–PGL-I antibody is responsible for cross-reacting with leishmanial antigens. Keeping these interesting observations in mind, we next compared the reactivity of PGL-I with serum samples obtained from patients with visceral leishmaniasis (VL) and from patients with leprosy against the reactivity of PGL-I with serum samples obtained from healthy control subjects. In an experiment using an enzyme-linked immunosorbent assay experiment and the same serum samples shown in Figure 1*A*, we found that 50% of the VL serum samples showed positivity with PGL-I, although the antibody titer level was low (Figure 1*B*). Moreover, when leishmanial antigen–coated wells were incubated with different dilutions of mouse monoclonal antibody or rabbit polyclonal

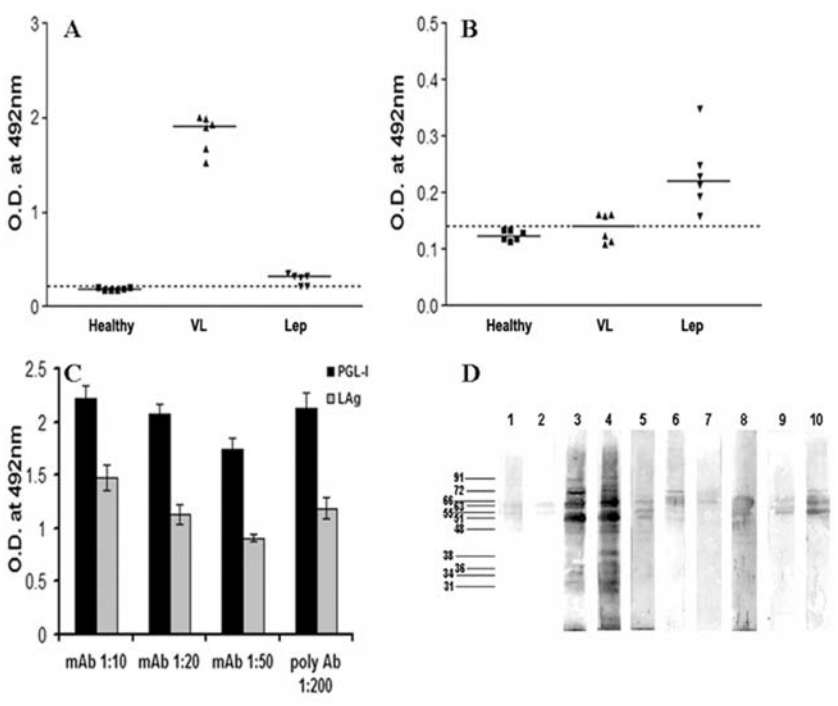

Figure 1 Recognition of leishmanial antigen and phenolic glycolipid–I (PGL-I) in the serum samples of patients with visceral leishmaniasis (VL) and patients with leprosy (Lep) and in leprosy-specific antibodies. Panels A and B show the reactivity of the serum samples of healthy control subjects, patients with VL, and patients with leprosy (1:500 dilutions) against the *Leishmania donovani* antigen (LAg) [3] and the antigen PGL-I* of *Mycobacterium leprae*, respectively. The dotted lines show the cutoff value (mean of healthy control subjects + [2 × standard deviation]), and the solid lines show the median of each group. Panel C shows the optical density (O.D.) (± standard error) of the reactivity of PGL-I and LAg with monoclonal* (mAb) and polyclonal* (poly Ab) antibodies to PGL-I (antibody dilutions are indicated in the figure). Panel D shows recognition of bands of LAg (6 μg/lane) by serum samples diluted to 1:100 from healthy individuals *(lanes 1–2),* patients with VL *(lanes 3–4),* patients with leprosy *(lanes 5–6),* and healthy rabbit and mouse *(lanes 7 and 9),* a 1:100 dilution of rabbit poly Ab *(lane 8),* and a 1:500 dilution of mAb to PGL-I *(lane 10).* *Kind gifts from Dr. P. J. Brennan, Colorado State University.

antibody to PGL-I, a statistically significant ($P < .001$ at 95% confidence intervals) dilution-dependent antigen was recognized (Figure 1*C*), compared with normal mouse and rabbit serum samples, respectively (with optical density values <0.2).

We then performed the immunoblot assay to determine the specificity of the antibodies. Serum samples from healthy individuals, patients with VL, and patients with leprosy, as well as polyclonal and monoclonal antibodies to PGL-I (Figure 1*D*), were used to probe for leishmanial antigens [3]. A faint cross-reaction was observed to be restricted to 76–51 kDa bands among healthy control subjects. From the serum samples obtained from patients with VL, we recognized at least 11 polypeptides. Serum samples obtained from patients with active leprosy also demonstrated a cross-reaction with leishmanial antigens present in the 72, 63, 55, and 51 kDa bands. Interestingly, we observed 3 distinct bands of 72, 63, and 55 kDa being recognized by the monoclonal antibody specific for PGL-I. These polypeptides were abundantly recoginized by serum samples of patients with kala-azar as well [4]. The rabbit polyclonal antibody against PGL-I showed a blotch in the region between 72 and 55 kDa. Therefore, the polyclonal antibody recognizes the same polypeptides as does the monoclonal antibody. These 3 distinctly different molecular weight antigens of *Leishmania* somehow share the same exposed antigenic determinants to form the molecular configuration of PGL-I. Healthy mouse and rabbit serum samples were used as controls.

Our finding therefore reveals that PGL-I is not a specific antigen for *M. leprae*, as claimed by Spencer et al [5], at least in the Indian scenario, because it cross-reacts with leishmanial antigens. This is a very important finding, because many laboratories are trying to establish diagnostic tests using this antigen for detection of early leprosy [6].

Acknowledgments

We thank Prof. Patrick J. Brennan of Colorado State University for providing PGL-I and for providing polyclonal and monoclonal antibodies to PGL-I from his laboratory.

Potential conflicts of interest. All authors: no conflicts.

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Clinical Infectious Diseases 2010; 50:937–938

1058-4838/2010/5006-0026$15.00
DOI: 10.1086/650541

Cover Image and Description for the 1 November 2009 Issue

To the Editor—In their comment on the cover art ("A Leper with a Bell," vellum, English School [15th century]) for the 1 November 2009 issue of *Clinical Infectious Diseases*, Grizzard and Grizzard state that "the bell would have been important to make the leper heard while begging for alms" as a result of vocal cord damage from advanced leprosy [1]. Although this may be part of the story of the illustration, probably much more important was the requirement that individuals with leprosy carry a clapper or bell to warn people they were approaching so that they could avoid others (as they were required to do) and others could avoid them. This was part of the process of making the person an outcast. Although vocal cord involvement certainly can occur in leprosy, I believe it unlikely that this was responsible for the widespread use of clappers and bells by those with leprosy [2].

This process of depriving persons with leprosy of their humanity and rights contributed greatly to the stigma that still accompanies this disease and contributes to the strong aversion of persons with leprosy (or Hansen disease) to use of the term *leper*. I believe it should not have been used in this commentary in the journal. If authors feel it necessary to use the word *leper* for historical purposes, I suggest that it be placed in quotation marks.

Acknowledgments

Potential conflicts of interest. R.I.F.: no conflicts.

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Clinical Infectious Diseases 2010; 50:938

1058-4838/2010/5006-0027$15.00
DOI: 10.1086/650741