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Antibodies to Phenolic Glycolipid-I and Sulfatide-I in Leprosy and Tuberculosis

TO THE EDITOR:

Following the discovery of phenolic glycolipid-I (PGL-I) by Hunter and Brennan ([11]) in *Mycobacterium leprae*-infected tissues and once being established that the material was specific to *M. leprae* ([12]), several assays for the detection of that lipid antigen have been developed with the intention of applying them in the serological diagnosis of leprosy ([5, 18]) to identify those household contacts with an incipient disease ([4]) and to monitor the response of the patients subjected to chemotherapy ([2]). A similar glycolipid has been isolated and characterized from *M. tuberculosis* by Daffe, *et al.* ([7]) and has been used by some authors for the serological diagnosis of tuberculosis with variable results ([3, 15, 16]).

In this study, we measured the reactivity of the sera from 34 tuberculous patients, 33 patients with lepromatous leprosy, and 38 healthy individuals to PGL-I of *M. leprae* and to sulfolipid-I of *M. tuberculosis* H37Rv. Each lipid has been considered to be species-specific, and in the case of PGL-I, this specificity has been the basis for its use as an antigen for the serological diagnosis of leprosy. Although a similar consideration of specificity has been given to the sulfolipid-I (sulfatide-I, SL-I) of *M. tuberculosis*, its use as an antigen for the diagnosis of tuberculosis has not been a common practice, perhaps because of the more extensive information on protein antigens ([1, 9, 14]) and other lipids ([6–8]). PGL-I was isolated from *M. leprae*-infected armadillo tissue by the techniques of Vemuri, *et al.* ([17]) and Hunter, *et al.* ([13]). SL-I was purified from *M. tuberculosis* H37Rv using the method of Goren, *et al.* ([10]). Although the patients studied were under treatment at the time of sampling and most leprosy patients were old multitreated cases, all of the patients still had active disease. Patients and control groups included both male and female individuals whose ages ranged from 16 to 72 years.

Antibodies to the mycobacterial lipids were measured using an enzyme-linked immunosorbent assay (ELISA) adapted for lipid antigens. From the results, it could be concluded that: a) lepromatous (LL) sera and tuberculous (Tb) sera contain similar amounts of IgG antibodies to PGL-I [0.154 ± 0.101 (mean OD 492 nm of triplicates $\pm$ S.D.) in LL vs 0.104 ± 0.052 in Tb, $p = 0.5$]; b) LL sera contain higher levels of IgM antibodies to PGL-I than Tb sera (0.164 ± 0.227 vs 0.046 ± 0.035, respectively; $p = 0.01$); c) LL sera and Tb sera show similar amounts of IgG antibodies to SL-I (0.144 ± 0.072 vs 0.096 ± 0.050, $p = 0.5$); d) LL sera and Tb sera contain similar, very low amounts, if any, of IgM antibodies to SL-I (0.019 ± 0.034 vs 0.026 ± 0.020, respectively; $p = 0.5$), and e) although low, the levels of IgG and IgM antibodies to PGL-I and to SL-I in LL and Tb sera were still higher than those levels in the control group, with the numerical values not always reaching statistical significance.

THE TABLE. *Antibodies to PGL-I and SL-I in the sera of normal (NL), leprous (LL), and tuberculous (Tb) individuals.*[a]

	PGL-I antigen			SL-I antigen		
Antibody isotype	NL	LL	Tb	NL	LL	Tb
Total Igs	0.181 ± 0.128	0.609 ± 0.244	0.472 ± 0.235	0.277 ± 0.030	0.472 ± 0.142	0.488 ± 0.161
IgG	0.059 ± 0.036	0.154 ± 0.101	0.104 ± 0.052	0.067 ± 0.033	0.019 ± 0.034	0.096 ± 0.050
IgM	0.021 ± 0.007	0.164 ± 0.227	0.046 ± 0.035	0.009 ± 0.001	0.019 ± 0.034	0.026 ± 0.020

[a] Average readings (492 nm) of triplicate determinations; means and standard deviations are shown.

These results may indicate a) that *M. leprae* contain antigens with epitopes related to SL-I, b) that some antigen(s) in *M. tuberculosis* may share one or more epitopes with the PGL-I of *M.* leprae, or c) that leprosy patients are (or were) also infected by *M. tuberculosis,* with this infection not necessarily overt but subclinical. The third possibility seems to be more likely since antibodies to SL-I were also found in several healthy subjects, a fact that indicates a previous or present contact with the microorganism (in Mexico, tuberculosis is endemic and a high portion of the population is PPD+).

Taken together, the results show that a variable degree of crossreactivity with PGL-I and SL-I is detected in the serum of patients with leprosy and/or tuberculosis. This makes the use of these lipid antigens inadequate for the differential diagnosis of these mycobacterioses (more so in geographic areas where one or the two mycobacterioses are endemic).

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NADPH-Oxidase Activity Triggered by Endocytosis of Yeast in Circulating Phagocytes of Nine-Banded Armadillos (*Dasypus novemcinctus*)

TO THE EDITOR:

Dasypus novemcinctus, the nine-banded armadillo, is regarded as a host for *Mycobacterium leprae* [7, 8, 12]. In those geographic areas where leprosy is endemic, wild armadillos have been considered potential natural reservoirs for the leprosy bacillus [10]. Despite its great importance as an alternative host for *M. leprae*, the immunobiology of the armadillo is an aspect still little explored. As in humans, armadillo *M. leprae* is also an intracellular parasite of macrophages; *M. leprae*-laden macrophages accumulate to form macrophagic granulomas, the characteristic lesions of leprosy. Granulomas may appear internally in the viscerae and more superficially in the skin and underlaying structures [1]. The fate of *M. leprae* in the armadillo is very likely dependent on the function of macrophages (modulated by cell-mediated immunity), and early in the infection it is probably also dependent on the function of polymorphonuclear (PMN) leukocytes. Macrophages and PMN leukocytes are professional phagocytic cells of the body whose combined function accounts for the killing of invading microorganisms; killing by phagocytic cells depends, in turn, on a variety of mechanisms of which some are oxygen-dependent and some are not [11]. Oxygen-dependent mechanisms include the generation of highly reactive oxygen species (free radicals) as well as the activity of the myeloperoxidase system [9]. Among the microbicidal mechanisms that do not depend on oxygen are the acidic milieu within the phagolysosomes, the hydrolytic activity of neutral and acidic hydrolases, the effect of cationic proteins other than hydrolytic enzymes, and the activity of factors that increase the permeability of bacterial cell walls (BPIFs). An additional microbicidal mechanism, this one strongly exerted by immunologically activated macrophages, depends on the metabolism of arginine in which nitric oxide is an important byproduct [5]. As oxygen metabolites (superoxide anion, hydroxyl radicals and oxygen singlet), nitric oxide is a highly unstable species and thus an extremely reactive radical. Oxygen metabolites and nitric oxide are able to chemically modify the microbial body, arresting its metabolism, leaving the microorganism inert and unable to repair