Serologic Assays Using the 18-kDa Antigen of *Mycobacterium leprae* Expressed in the Yeast *Saccharomyces cerevisiae*

TO THE EDITOR:

Leprosy diagnosis is mainly based on clinical data and is confirmed by the detection of *Mycobacterium leprae* in infected tissues by direct bacilloscopy. In tuberculoid (TT) cases, the bacilli are seldom found and the diagnosis can only be confirmed by histopathological examination. The development of specific serological assays for easier etiological diagnosis would allow the realization of seroepidemiological assays and would improve the follow up of patients, which possibly would imply in the installation of earlier and more effective control measures ([8]).

Among different *M. leprae* antigens, special attention was given to the 18-kDa antigen (p18), since it was involved in the immune response in mice and in humans ([4, 7]) and was initially considered restricted to two mycobacteria species, because cross-reaction using a specific monoclonal antibody was only found with *M. simiae* serovar 1 ([12]). Our group have developed a system for the expression of p18 in *Saccharomyces cerevisiae* utilizing an inducible promoter and a secretion cassette that produces more than 100 mg/L of p18 ([18]). This antigen was purified and used for delayed-type hypersensitivity assays in mice ([19]). In the present report, the use of this purified recombinant antigen in enzyme immunoassays (ELISA) was verified for the detection of *M. leprae* infections.

We studied seven leprosy outpatients followed up at the Department of Dermatology from São Paulo State Health Secretary at São Paulo, Brazil. The bacterial index (BI) (1+–5+) was determined by analysis of the earlobe exudate and the clinical form was classified according to the criteria proposed by Ridley and Jopling ([20]). For our control group, we used eight confined individuals from a psychiatric hospital at Juquerí (about 30 km from São Paulo) for whom no previous contact with leprosy patients had been reported. The Brazilian Ministry of Health ethics guidelines were followed.

RESULTS AND DISCUSSION

Converse to previous serological assays, in this study we analyzed the use of an antigen corresponding to the authentic p18 expressed in *S. cerevisiae* for the detection of specific antibodies. This study is also the first to analyze the detection of anti-p18 antibodies among Brazilian patients.

The results of the ELISAs for the detection of anti-p18 antibodies (total and classes IgG, IgM and IgA) in the leprosy patients and the control group are shown in The Table. For total antibodies, IgA and IgM classes, patient sera were diluted 1/10.

For the IgG class, serum dilution ranged from 1/100 to 1/1000. The means of the OD_{492} values obtained from leprosy patients were always higher than those obtained from the control group, independent of the antibody class analyzed, but no statistically significant differences were found (Student's *t* test). The largest difference between the mean value in patients and in the control group was obtained when the patient sera was diluted 1/1000. Some individuals in the control group also showed higher OD_{492} values.

Other studies have shown that p18 would not be suitable for a routine serological assay for the diagnosis or follow up of leprosy patients since many patients (especially at the tuberculoid pole) did not show anti-p18 antibodies but some control patients also showed these antibodies ([9–11, 21, 22]).

These previous results led us to first check only patients closer to the lepromatous pole of the disease. We observed that the average levels of antibodies against p18 in these patients were generally higher than those of the control group. However, some leprosy patients did not show anti-p18 anti-

THE TABLE. *OD_{492} values obtained with anti-P18 specific ELISA in sera tested at different dilutions.*

Patient	Clinical form	Bacterial index	IgG				Ig total	IgA	IgM
			1/1000	1/500	1/200	1/100	1/10	1/10	1/10
					Leprosy patients				
1	LL	3	0.279	0.433	0.768	1.546	0.304	0.069	0.148
2	LL	3	0.291	0.377	0.639	1.367	2.232	0.154	0.334
3	LL	0	0.451	0.645	1.107	1.869	2.062	0.380	0.161
4	BB	2	0.126	0.142	0.330	0.748	1.333	0.159	0.082
5	LL	3	0.079	0.138	0.254	0.585	0.507	0.081	0.103
6	BL	5	0.084	0.220	0.402	0.815	1.647	0.200	0.104
7	LL	3	0.070	0.105	0.167	0.225	0.955	0.052	0.115
Mean ± S.D.			0.197 ± 0.135	0.294 ± 0.184	0.524 ± 0.308	1.022 ± 0.542	1.292 ± 0.688	0.156 ± 0.104	0.150 ± 0.079
					Controls				
8			0.116	0.360	0.842	1.835	2.484	0.276	0.275
9			0.097	0.308	0.629	1.467	0.340	0.061	0.061
10			0.053	0.086	0.179	0.345	0.825	0.045	0.052
11			0.088	0.308	0.627	1.381	0.911	0.104	0.107
12			0.078	0.141	0.256	0.367	0.655	0.076	0.087
13			0.063	0.096	0.159	0.280	0.842	0.109	0.074
14			0.056	0.113	0.258	0.186	0.871	0.091	0.089
15			0.055	0.087	0.133	0.757	1.493	0.145	0.131
Mean ± S.D.			0.076 ± 0.022	0.187 ± 0.109	0.385 ± 0.254	0.827 ± 0.601	1.053 ± 0.848	0.113 ± 0.090	0.110 ± 0.086

bodies and some individuals in the control group did have high antibodies levels against p18.

Initial reports describe this antigen restricted to a few mycobacterial species, such as *M. simiae* serovar 1 ([12]) and *M. smegmatis* ([15]). Later, it was found that crossreaction could also be found with *M. tuberculosis* and *M. bovis* BCG ([5, 6, 21, 22]). These data were not confirmed by other authors, especially at the T-cell level ([16, 17, 18]), and no homologous gene was found in *M. tuberculosis* even when using polymerase chain reaction (PCR) with degenerated primers ([3]). Interestingly, the presence of T-cell clones recognizing specifically p18 from *M. leprae* (but not from other mycobacteria species) was recently reported from one subject who was not exposed to leprosy ([1]). The nature of the antigen stimulating this apparent specific response could not be determined.

On the other hand, the absence of detectable anti-p18 antibodies in some leprosy patients can be explained by the fact that the response against this protein in the natural infection seems to be more important at the cellular level ([17]) and is not an early event ([13]).

It is noteworthy that the patient who showed the highest antibody levels for all of the immunoglobulins tested (except IgM, for which he showed the second highest antibody level tested) was also the only one analyzed who showed a negative result on the bacilloscopy assay, although he was classified as LL. This result agrees with some data previously published suggesting that p18 would elicit a protective response against *M. leprae* infection ([6]). It was recently shown that immunization with recombinant BCG expressing p18 was capable of inducing antibodies, a lymphocyte proliferative response, and some protection against infection ([2]).

In conclusion, the 18-kDa recombinant antigen of *M. leprae* obtained in *S. cerevisiae* does not seem useful for routine serological assays for the diagnosis or follow up of leprosy patients.

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Collagen Profile in Sciatic Nerves of *M. leprae*-Inoculated Mice Correlates With *in vitro* Collagen Production by Schwann Cells

To the Editor:

Peripheral nerves are tissues especially rich in their collagen content, with the prominent collagen types being I, III, IV and V ([8]). Histopathological observations of nerves from leprosy patients reveal increased collagen deposition in the early stages and the inflammatory cell populated later stages ([6, 7]). Sciatic nerves from experimentally infected Swiss white (SW) mice also show the presence of collagen pockets, indicative of freshly laid down matrix, around unmyelinated fibers ([9, 10]). This has been corroborated by *in vitro* studies where Schwann cells, the major producer of collagen in the peripheral nerve ([5]), from SW mice have been shown to produce increased levels of different collagen types on infection with *Mycobacterium leprae* ([11]). The SW mouse is a strain in which the response of host cells to *M. leprae* infection parallels those observed in lepromatous patients ([3, 4]) as opposed to the C57BL/6 mouse, a strain in which the response to *M. leprae* parallels that observed in tuberculoid patients or normal individuals ([3, 4]). *In vitro,* Schwann cells from C57BL/6 mice exhibit unaltered collagen metabolism on infection with *M. leprae* ([11]). Expression of collagen types I, III, and IV, the most abundant collagen types found in peripheral nerves, was therefore studied using indirect immunoperoxidase staining to determine its correlation with *in vitro* observation on collagen production by neural cell population.

The SW and C57BL/6 strains of mice were inoculated with 10^4 *M. leprae*/foot pad. Sciatic nerves were collected from the animals at months 12 and 20 postinfection. At each time interval, sciatic nerves from age-matched, uninfected control mice were also collected. The nerves were placed in Formal-Zenker fixative and processed for paraffin blocks. Slides containing transverse and longitudinal nerve sections (5-μm thick) were dewaxed by treating with xylene. The sections were rehydrated by passing through graded ethanol and treated with Lugol's iodine and sodium thiosulfate. The sections were then treated with 3% hydrogen peroxide for 15 min for nonspecific peroxidase and preblocked with 1% fetal calf serum for 30 min at 37°C. The sections were then treated with a 1:50 dilution of antibodies raised in goat against human collagen types I and III (Sera-lab, code 1310 and 1330, respectively) for 3 hr at 37°C. Primary antibody minus sections for each nerve were included as negative controls. Following washes with TBS (Tris buffered saline containing 0.05% Tween), the sections were incubated with a 1:20 dilution of horseradish peroxidase conjugated anti-