Investigation of Anti-*Mycobacterium leprae* Antibodies in Leprosy Patients' Sera by an A60 Antigen Immunoassay

TO THE EDITOR:

Antigen 60 (A60) described by Cocito and Van Linden ([5]) in 1986 is a cytoplasmic antigen purified from *Mycobacterium bovis* BCG.

Several authors, including Ladron de Guevara, *et al.* ([6]) and Sanchez Monton and Martin Luengo ([7]), have employed A60 in the serodiagnosis of leprosy with different results.

The object of our study, in light of the limited existing studies on leprosy with A60 and given the relative important number of cases in our community, is to demonstrate the presence of immunoglobulin G (IgG) against A60, as well as the possible utility of this technique in the diagnosis of leprosy.

A total of 121 leprosy patients were studied and classified according to the Riddley-Jopling criteria as follows: 90 lepromatous leprosy (LL), 7 borderline lepromatous (BL), 6 borderline borderline (BB), 1 borderline tuberculoid (BT) and 17 tuberculoid (TT) leprosy. The study also included 23 immediate family members. Neither patients nor healthy contacts included in our study had been previously vaccinated for tuberculosis. Those individuals included as healthy contacts and leprosy patients had

tion or active disease. The majority of the sera studied were from Cordoba and its province. The Sanitorium of San Francisco de Borja of Fontilles supplied 10 sera which had come from other provinces in Spain.

An indirect ELISA technique using the commercialized Anda-Tb test kit ([4]) was employed in our study.

Of the 90 LL patients, 43% were positive, 15% intermediate, and 42% negative. Of the 7 BL, 71% were positive and 29% intermediate. Of the 6 BB, 50% were positive, 33% intermediate and 17% negative. The only BT patient was negative. Of the 17 TT, 18% were positive, 6% intermediate and 76% negative. We found significant statistical differences in the proportion of positivity in the different clinical forms of leprosy ($p < 0.01$).

With respect to the detection of IgG in the 23 family members of leprosy patients, 16 were negative, 3 intermediate and 4 positive (of which one had high levels of positivity).

The ELISA was positive in 41% of the patients and in 17% of the family members. There was an intermediate response in 15% of the patients and 13% of the family members; while 44% of the patients and 70% of the family members had negative responses. These results were statistically significant ($p < 0.001$).

In a study by Ladron de Guevara, *et al.* ([6]), 20.8% of the clinically healthy contacts had antibodies against A60, with 12.5% being very positive. This level of positivity is very similiar to that observed by other authors such as Buchanan, *et al.* ([3]) in 1983 against phenolic glycolipid-I (PGL-I) in family members of patients in Mexico, in which leprosy is as endemic as in Spain. In highly endemic countries the levels are higher ([1, 2]).

We observed also a direct relationship between antibody levels and bacillary load ($p < 0.0l$).

We conclude that the detection of IgG against A60 by the ELISA could be useful as a complementary test in the diagnosis of leprosy, especially in suspected cases of lepromatous leprosy. Independent of the clinical form, a direct relationship exists between the presence of anti-IgG antibodies against A60 and the bacillary load. A prospective study on the evolution of family members with an immunologic status of lepromatous leprosy (Mitsuda negative and ELISA positive) would be of value in that family members are at high risk of developing the disease.

—Manuel Casal, M.D.
Francisco Solis, D.Pharm.
M. Jose Linares, D.Biol.
Concepcion Puig, M.D.

Department of Medical Microbiology
Faculty of Medicine
Cordoba University
Avda. Menendez Pidal, S/N
14004 Cordoba, Spain

Reprint requests to Dr. Casal at the above address or FAX 34-957-218229.

Acknowledgment. The authors gratefully thank Dr. Terencio de las Aguas, Director of the Sanatorium of San Francisco de Borja of Fontilles, and Pilar Font of the Department of Statistics, Faculty of Medicine, Córdoba University, for their contributions.

REFERENCES

1. Agis, F., Schlich, P., Cartel, J. L., Guidi, C. and Bach, M.-A. Use of anti-*M. leprae* phenolic glycolipid I antibody detection for early diagnosis and prognosis of leprosy. Int. J. Lepr. **56** (1988) 527–535.
2. Baumgart, K., Britton, W., Basten, A. and Bagshawe, H. Use of phenolic glycolipid I for

serodiagnosis of leprosy high prevalence village in Papua New Guinea. Trans. R. Soc. Trop. Med. Hyg. **81** (1987) 1030–1032.

3. BUCHANAN, T. M., YOUNG, D. B., MILLER, R. A. and KHANOLKAR, S. R. Serodiagnosis of infection with *Mycobacterium leprae*. Int. J. Lepr. **51** (1983) 524–530.
4. CASAL, M., SOLÍS, F., LINARES, M. J., ARIAS, M. C., REDONDO, J. M. and PUIG, C. The use of ELISA with antigen 60 in a serological study of human tuberculosis. (Seiter) Eur. J. Med. **2** 199–318.
5. COCITO, C. and VAN LINDEN, F. Preparation and properties of antigen 60 from Mycobacterium bovis BCG. Clin. Exp. Immunol. **66** (1986) 262–272.
6. LADRON DE GUEVARA, M. C., BELTRAN, M., MARTIN, V., FERNÁNDEZ R. and SANZ, J. V. Anticuerpos frente al antígeno A 60 en enfermos de lepra y contactos. Rev. Esp. Microbiol. Clin. **6** (1991) 337–340.
7. SÁNCHEZ MONTÓN, T. and MARTÍN LUENGO, F. Serodiagnóstico de la tuberculosis pulmonar: valoración clínica. Enf. Infec. Microbiol. Clin. **10** (1992) 267–271.