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Humoral Responses against the 85A and 85B Antigens of *Mycobacterium bovis* BCG in Patients with Leprosy and Tuberculosis

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Immunoglobulin G antibodies against the 85A and 85B components of the *Mycobacterium bovis* BCG antigen 85 complex separated by isoelectric focusing were investigated in serum samples from 129 patients representing the major forms of leprosy, 111 tuberculous patients, and 153 healthy subjects. For both of the antigens, a higher degree of staining was observed for lepromatous leprosy patients and patients with active tuberculosis than for the other groups. Because sera from some healthy subjects recognized the 85A antigen, we suggest that antigen 85B is the most useful component of the antigen 85 complex for the serodiagnosis of the multibacillary forms of leprosy or of the active forms of tuberculosis.

Tuberculosis and leprosy are caused by mycobacteria and remain major diseases in developing countries. Laboratory diagnosis of tuberculosis can be difficult: microscopic examination gives a low positive yield, whereas culture techniques require complex media and sometimes fail to detect the disease sufficiently early (9).

The spectrum of activity of leprosy is very broad, and it is the location of a patient in this spectrum that determines his or her infectivity and long-term prognosis (12). Therefore, it is essential to accurately classify disease activity.

The development of the enzyme-linked immunosorbent assay (4) and of the dot-immunobinding assay (14) using mycobacterial antigens has stimulated interest in serological tests for the diagnosis of mycobacterial infections. For tuberculosis, the antibody response has been studied extensively; for leprosy, the investigation of the specific humoral response has been hampered by the difficulty in cultivating the leprosy bacillus in vitro and in obtaining purified antigens. Most of the serological assays to differentiate healthy contacts from leprosy patients and to identify these leprosy patients within the widely accepted Ridley-Jopling classification therefore involved use of glycolipids (2).

An alternative approach was based on utilization of identical proteins for the serodiagnosis of tuberculosis and of leprosy, *Mycobacterium leprae* and *Mycobacterium tuberculosis* having many antigens in common (5). These antigens include the so-called 29/33-kDa protein doublet, which corresponds to the secreted (6) and fibronectin-binding (1) antigen 85 complex (3–10).

We recently developed a simple isoelectric focusing technique to separate the components of the complex (8). In this paper, we report on the Western blot (immunoblot) analysis of the humoral response directed against the 85A and 85B components in large numbers of tuberculous patients as well as of leprosy patients, of contacts, and of control subjects.

Sixty-five purified protein derivative- or lepromin skin test-negative healthy volunteers, 34 purified protein derivative-skin test-positive individuals who had received *Mycobacterium bovis* BCG vaccination, and 54 healthy household contacts of leprosy patients (24 lepromin skin test positive) were studied. One hundred twenty-nine serum samples were collected from leprosy patients. All patients either were untreated or had started therapy less than one month earlier. They were classified according to the Ridley-Jopling criteria into polar lepromatous (LL; $n = 42$), borderline lepromatous (BL; $n = 28$), borderline tuberculoid (BT; $n = 20$), and polar tuberculoid (TT; $n = 39$) leprosy.

One hundred eleven serum samples from patients with *M. tuberculosis* infection were tested. Diagnosis was based on microscopic examination and/or culture. Of these serum samples, 82 were obtained before treatment and 29 were obtained at least 2 months after the beginning of treatment.

The antigen 85 complex was purified by sequential phenyl-Sepharose and DEAE-Sephacel chromatography from a 14-day-old *M. bovis* BCG (Pasteur reference strain 1173P2) culture filtrate (8).

Vertical nondenaturing isoelectric focusing was performed in a 2.5 to 6.5 pH gradient (8). Samples, each containing 3 μg of the unseparated BCG antigen 85 complex per cm of gel (in width), were added at the cathode. The proteins were next electrophoretically transferred onto nitrocellulose sheets (0.2 μm; Bio-Rad Laboratories). Nitrocellulose strips were incubated overnight with human serum samples diluted in Tris-buffered saline–0.05% Tween (TBS-T) containing 1% bovine serum albumin, washed in TBS-T, and incubated for another 4 h with peroxidase-conjugated anti-human immunoglobulin G (IgG) rabbit immunoglobulins (Dakopatts, Copenhagen, Denmark) diluted 1/500 in TBS-T. After rinsing the strips in TBS-T, staining was performed by the addition of peroxidase substrate (Bio-Rad Laboratories) containing alpha-chloronaphthol in the presence of hydrogen peroxide (8). The optimal working dilution, 1:100, was established by titration of serum samples from healthy subjects, tuberculosis patients, and leprosy patients. The reactivity of the sera against the antigen 85 complex was tested twice. Control

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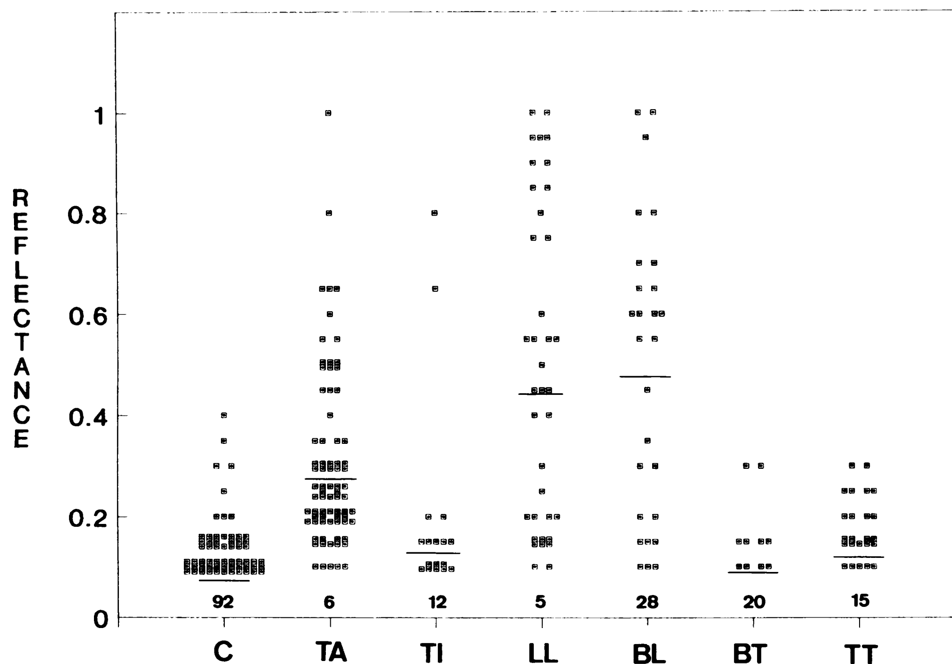

FIG. 1. IgG antibody response against antigen 85A. Shown are reflectances for control subjects (C; n = 153) and for patients with active tuberculosis (TA; n = 82), inactivated tuberculosis (TI; n = 29), lepromatous leprosy (LL; n = 42), borderline lepromatous leprosy (BL; n = 28), borderline tuberculoid leprosy (BT; n = 20), and tuberculoid leprosy (TT; n = 39). For each group, the number of sera with reflectance values below the detection limit is given at the bottom of each column. The mean reflectance for each group is indicated by a horizontal line (——).

sera of well-known reactivity were included in each run to verify the results.

The purified antigen 85 complex used in the present study contains a higher proportion of antigen 85B than the preparations previously described (8, 13), antigen 85A remaining the more abundant component as estimated by gel scanning (60% of the total protein amount) after Coomassie blue staining.

After transfer to nitrocellulose, antigens 85A, 85B, and 85C were stained to various degrees by the sera. The degree

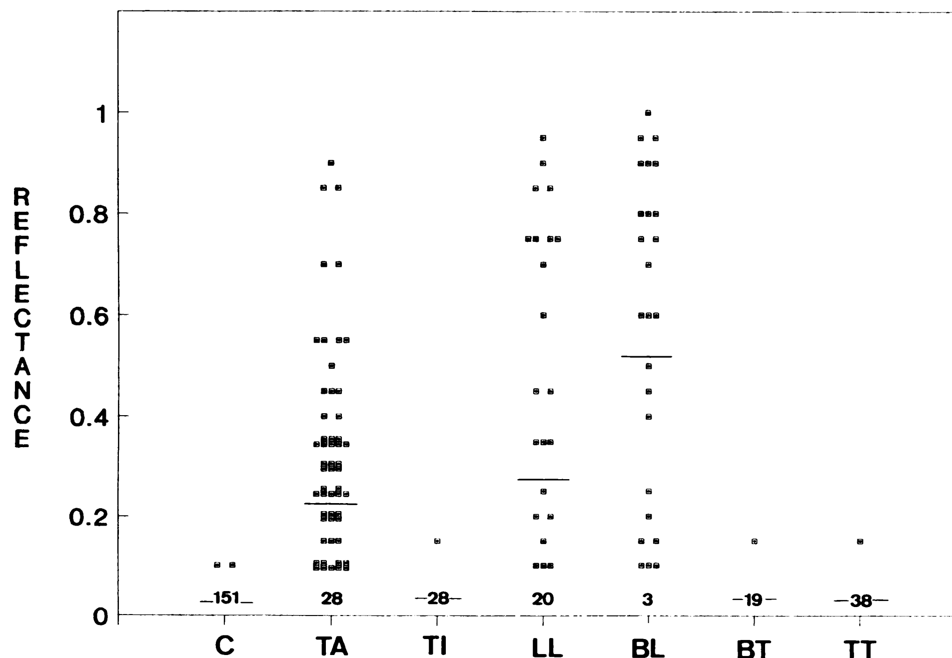

FIG. 2. IgG antibody response against antigen 85B. For an explanation of symbols, see the legend to Fig. 1.

of staining of the protein bands was quantified (8) by determination of integrated reflectance values (expressed in color yield units) with the Bio-Rad model 620 video densitometer. Figures 1 and 2 summarize the reflectance values of the antigen 85A and 85B bands for the 393 serum samples tested. The degree of staining for antigen 85C was by and large too low for quantitative determination.

Antigen 85A was recognized by the sera of some healthy subjects independently of skin test reactivity, previous BCG vaccination, or contact with leprous individuals. The highest reflectances were measured with either 85A or 85B antigens when testing serum samples from BL and LL patients. The data confirmed the observations of Pessolani et al. (11), who, using the purified 85A and 85B components in an enzyme-linked immunosorbent assay, recently demonstrated that the antigen 85B provided the best distinction between lepromatous and tuberculoid leprosy patients. The high bacterial load in sera from untreated LL patients and entrapment of specific antibodies into immune complexes possibly explain why lower reflectance values were obtained for LL leprosy patients than for BL leprosy patients.

The reflectances measured for tuberculous patients were lower than those for leprosy patients but higher than those for control subjects. As was found earlier (7), by using either the 85A or the 85B antigens, the humoral response is much higher for patients with active tuberculosis than for those with inactivated forms of the malady. This could explain why Pessolani et al. (11) did not observe an increase in levels of specific antibodies against antigens 85A and 85B for the tuberculous patients whom they investigated, who were actually all undergoing treatment.

Our observations show the importance of choosing antigens inducing a specific humoral response evolving in parallel with the disease activity when developing a serodiagnostic test of tuberculosis.

In conclusion, our study shows the possible diagnostic value of an IgG immune response against the 85B component of the BCG antigen 85 complex for detecting active tuberculosis and the early forms of multibacillary leprosy.

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REFERENCES

1. **Abou-Zeid, C., T. L. Ratliff, H. G. Wiker, M. Harboe, J. Bennedsen, and G. A. W. Rook.** 1988. Characterization of fibronectin-binding antigens released by *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG. Infect. Immun. **56:** 3046–3051.
2. **Chanteau, S., J. L. Cartel, J. Roux, R. Plichart, and M. A. Bach.** 1988. Comparison of synthetic antigens for detecting antibodies to phenolic glycolipid 1 in patients with leprosy and their household contacts. J. Infect. Dis. **157:**770–776.
3. **Closs, O., M. Harboe, N. H. Axelsen-Christensen, and M. Magnussen.** 1980. The antigens of Mycobacterium bovis, strain BCG, studied by crossed immunoelectrophoresis: a reference system. Scand. J. Immunol. **12:**249–263.
4. **Daniel, T. M., and S. Debanne.** 1987. The serodiagnosis of tuberculosis and other mycobacterial diseases by ELISA. Am. Rev. Respir. Dis. **135:**1137–1151.
5. **Das, P. K., A. Rambukkana, J. G. Baas, D. G. Groothuis, and M. Halperin.** 1990. Enzyme-linked immunosorbent assay for distinguishing serological responses of lepromatous and tuberculoid leprosies to the 29/33-kilodalton doublet and 64-kilodalton antigens of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **28:**379–382.
6. **De Bruyn, J., K. Huygen, R. Bosmans, M. Fauville, R. Lippens, J. P. Van Vooren, P. Falmagne, M. Weckx, H. G. Wiker, M. Harboe, and M. Turneer.** 1987. Purification, partial characterization and identification of the 32 kDa-protein antigen of Mycobacterium bovis BCG. Microb. Pathog. **2:**351–360.
7. **Drowart, A., K. Huygen, J. De Bruyn, J. C. Yernault, C. M. Farber, and J. P. Van Vooren.** 1991. Antibody levels to whole culture filtrate and to purified P32 during treatment of smear positive tuberculosis. Chest **100(3):**685–687.
8. **Drowart, A., P. Launois, M. De Cock, K. Huygen, J. De Bruyn, J. C. Yernault, and J. P. Van Vooren.** 1991. An isoelectric focusing method for the study of the humoral response against the antigen 85 complex of Mycobacterium bovis BCG in the different forms of leprosy. J. Immunol. Methods **145:**223–228.
9. **Lowell, A. M.** 1984. Tuberculosis: its social and economic impact and some thoughts on epidemiology, p. 1021–1056. *In* P. Kubica and L. G. Wayne (ed.), The mycobacteria. Part B. Marcel Dekker, New York.
10. **Nagai, S., H. G. Wiker, M. Harboe, and M. Kinomoto.** 1991. Isolation and partial characterisation of major protein antigens in the culture fluid of *Mycobacterium tuberculosis*. Infect. Immun. **59:**372–379.
11. **Pessolani, M. C. V., J. M. Peralta, F. D. Rumjanek, H. M. Gomes, M. A. de Melo Marques, E. C. C. Almeida, M. H. F. Saad, and E. N. Sarno.** 1991. Serology and leprosy: immunoassays comparing immunoglobulin G antibody responses to 28- and 30-kilodalton proteins purified from *Mycobacterium bovis* BCG. J. Clin. Microbiol. **29:**2285–2290.
12. **Ridley, D. S., and W. H. Jopling.** 1966. Classification of leprosy according to immunity. A five group system. Int. J. Lepr. **34:**255–273.
13. **Van Vooren, J. P., A. Drowart, M. De Cock, A. Van Onckelen, M. H. d'Hoops, J. C. Yernault, C. Valcke, and K. Huygen.** 1991. Humoral immune response of tuberculosis patients against the three components of the *Mycobacterium bovis* BCG complex separated by isoelectric focusing. J. Clin. Microbiol. **29:**2348–2350.
14. **Van Vooren, J. P., M. Turneer, J. C. Yernault, J. De Bruyn, E. Burton, F. Legros, and C. M. Farber.** 1988. A multidot immunobinding assay for the serodiagnosis of tuberculosis, comparison with an enzyme linked immunosorbent assay. J. Immunol. Methods **113:**45–49.