JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1988, p. 2200–2202
0095-1137/88/102200-03$02.00/0

Vol. 26, No. 10

Serological Responses of Patients with Lepromatous and Tuberculoid Leprosy to 30-, 31-, and 32-Kilodalton Antigens of *Mycobacterium tuberculosis*

HELLA S. RUMSCHLAG,* THOMAS M. SHINNICK, AND MITCHELL L. COHEN

Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

Received 17 June 1988/Accepted 5 July 1988

Sera from patients with lepromatous and tuberculoid leprosy were examined in immunoblot assays for antibodies to *Mycobacterium tuberculosis* culture filtrate antigens. Antibodies to 30- and 31-kilodalton proteins were present in 88 and 81%, respectively, of 16 patients with lepromatous disease and absent in 16 patients with tuberculoid disease. Antibodies to a 32-kilodalton protein were found in 12 and 38% of lepromatous and tuberculoid patients, respectively. These reactivities may be useful for distinguishing lepromatous and tuberculoid leprosy.

The forms of leprosy, as classified by Ridley, include the extremes of lepromatous and tuberculoid leprosy as well as a series of intermediate or borderline conditions (14). Lepromatous patients have abnormally high levels of antibody production and weak cell-mediated immunity, whereas tuberculoid patients have low antibody levels and strong cell-mediated immunity (11, 14). The basis of the variation in an individual's immune response to infection with *Mycobacterium leprae* and the subsequent manifestation of the disease are not yet understood. Some investigators suggest a link between human leukocyte antigen haplotype and the form of leprosy expressed (5, 9).

Recently, using an enzyme-linked-immunosorbent-assay-based antibody competition assay, Bothamley et al. (1) showed that antibodies reactive with *Mycobacterium tuberculosis* antigens were present in the sera of leprosy patients. This result was not surprising, since *M. leprae* and *M. tuberculosis* appear to share many antigens (6, 7, 13). Also, diseases caused by these mycobacteria are endemic in many of the same parts of the world. In this study, we examined the reactivity of sera from leprosy patients to unheated-culture filtrate antigens from *M. tuberculosis* in immunoblots. These investigations demonstrate that sera from patients with one form of the disease, lepromatous leprosy, react preferentially with certain protein antigens.

Sera from 16 lepromatous leprosy patients, 16 tuberculoid leprosy patients (Table 1), 20 tuberculosis patients, and 14 healthy individuals (6 from the World Health Organization IMMLEP Serum Bank and 8 from volunteers at the Centers for Disease Control) were tested for reactivity with *M. tuberculosis* culture filtrate antigens (4) by using an immunoblot procedure (3). Fifteen micrograms of the culture filtrate antigens was electrophoretically separated in each lane of sodium dodecyl sulfate–10% polyacrylamide gels by the discontinuous buffer system of Laemmli (10). Prestained molecular weight standards (Diversified Biotech, Newton-Centre, Mass.) were used as directed by the manufacturer. Proteins were electrophoretically transferred to nitrocellulose paper (BA85, 0.45-μm pore size; Schleicher & Schuell, Inc., Keene, N.H.) at 7 V/cm for 3 h in 25 mM Tris buffer (pH 8.6) containing 192 mM glycine and 20% (vol/vol) methanol (3). Unbound sites on the nitrocellulose paper were blocked by overnight incubation in casein buffer (10 mM Tris [pH 7.6], 154 mM NaCl, 0.5% casein, 0.02% thimerosal) (8). Casein buffer was also used as the diluent and washing agent in subsequent steps. Blot strips were incubated overnight at 4°C with heat-inactivated (54°C) human serum at a dilution of 1:50. After three 10-min washes, the strips were incubated with horseradish peroxidase-conjugated protein A (Bio-Rad Laboratories, Richmond, Calif.) at a dilution of 1:5,000 for 1 h. Bound conjugate was detected by development in TMB solution (per 100 ml: 25 mg of tetramethylbenzidine in 5 ml of methanol; 1.25 ml of 1 M Tris, pH 7.5; 20 ml of 5% dioctylsulfosuccinate, sodium salt; 0.025 ml of 30% H_2O_2).

General reactivity. Reactivity to antigens present in the *M. tuberculosis* culture filtrate was present in sera from 30 of 32 patients with leprosy, 18 of 20 patients with tuberculosis, and 5 of 14 healthy controls. Immunoblots of 18 serum samples from leprosy patients from Ethiopia as well as of representative tuberculosis and healthy control patients are shown in Fig. 1. In many cases, including the five healthy controls, reactivity was detected against a 32- to 55-kilodalton (kDa) smear which was resistant to proteinase K treatment. The sera from lepromatous patients (Fig. 1, samples 1 through 5) typically reacted more intensely and to more protein bands than did the sera from tuberculoid patients (Fig. 1, samples 9 through 18).

Specific reactivity. A different pattern of antibody reactivity was observed for three proteinase K-sensitive antigens with apparent molecular sizes of 32-, 31-, and 30-kDa (Fig. 1 and 2). Of the 16 serum samples from patients with lepromatous leprosy, 13 and 14 reacted with the 31- and 30-kDa antigens, respectively, and 2 reacted with a 32-kDa antigen. Of 16 serum samples from patients with tuberculoid leprosy, 6 bound the 32-kDa antigen, but none bound either the 31- or 30-kDa antigen (Table 1). Sera from five patients with polar tuberculoid leprosy did not react with any of the three antigens. Of the 20 serum samples from tuberculosis patients, 1 reacted with an antigen which comigrated with the 31-kDa antigen recognized by the sera from patients with lepromatous leprosy. None of the remaining 19 serum samples from tuberculosis patients and none of the 14 healthy control serum samples reacted with the 30-, 31-, or 32-kDa antigen.

M. leprae whole cells (from infected armadillos) were also

* Corresponding author.

TABLE 1. Occurrence of antibodies against specified antigens of *M. tuberculosis* culture filtrate in sera from patients with lepromatous and tuberculoid diseases

Disease classification (no. of samples)	No. (%) of samples positive for antigen of:		
	32 kDa	31 kDa	30 kDa
G. W. Long Hansen's Disease Center			
Lepromatous (9)	1 (11)	8 (89)	8 (89)
Tuberculoid (5)	0	0	0
W.H.O.[a]-Ethiopia			
Lepromatous (7)	1 (14)	5 (71)	6 (86)
Tuberculoid (11)	6 (54)	0	0
Totals			
Lepromatous (16)	2 (12)	13 (81)	14 (88)
Tuberculoid (16)	6 (38)	0	0

[a] W.H.O., World Health Organization.

examined by Western blot (immunoblot) analysis by using sera from a patient with lepromatous leprosy (LL 4). Proteins with apparent molecular weights of 30- and 31-kDa were detected (data not shown).

Discussion. The presence of antibody to *M. tuberculosis* antigens in patients with leprosy has been documented previously (1). The proteinase K-resistant 32- to 55-kDa smear often bound by sera of all classifications probably represents lipoarabinomannan, which has been shown to be conserved across bacterial species (6). The greater intensity of the reactions with sera from lepromatous patients may simply represent the higher antibody concentrations that are characteristic of this form of leprosy (12).

The surprising finding was the difference in the specificity of the sera from patients with tuberculoid and lepromatous diseases. In addition to a characteristic greater general reactivity, the sera from both American and Ethiopian patients with lepromatous disease were distinguished by specific antibodies to the 30- and 31-kDa proteins. The sera from patients with borderline tuberculoid disease did not react with these two proteins, but they often (6 of 11) reacted with the 32-kDa antigen.

Britton et al. (2) recently reported that antibodies present in the sera of patients with lepromatous leprosy (14 of 14

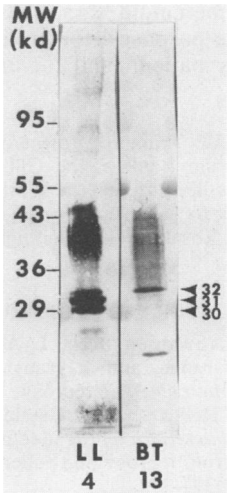

FIG. 2. Western blot strips of *M. tuberculosis* culture filtrate antigens reacted with human serum LL 4 (lepromatous) and BT 13 (borderline tuberculoid), emphasizing the differential reactivity to the 30-, and 31-, and 32-kDA antigens. Molecular weights are indicated on the left.

serum samples) but not tuberculoid leprosy (0 of 10 serum samples) precipitated antigens of 32- and 33-kDa from a radiolabeled *M. leprae* sonic extract. Also, a pool of sera from lepromatous leprosy patients was shown to precipitate similarly sized antigens from a radiolabeled sonic extract of *M. bovis* BCG. The data suggest that these antigens may correspond to the 30- and 31-kDa antigens described by us. If so, *M. tuberculosis* culture filtrates may be a readily available source of clinically useful amounts of these potentially immunodiagnostic antigens.

The specific nature of the binding of sera from leprosy patients to the 30-, 31-, and 32-kDa antigens indicates that further study of these proteins is warranted. Recent advances in the technology of recombinant DNA and monoclonal antibodies have facilitated the study of individual components of *M. leprae* without the need to grow large quantities of cells in animal models (15). These techniques could be used to evaluate the 30-, 31-, and 32-kDa antigens further. Considering the specificities of the reactions of

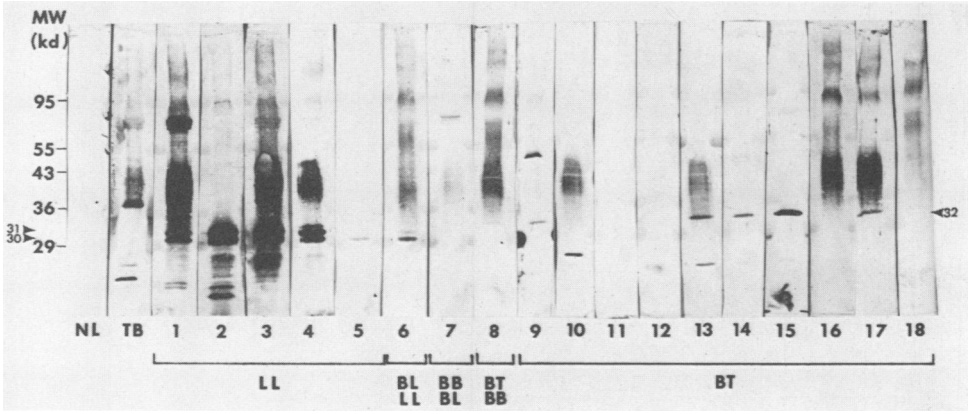

FIG. 1. Western blot strips of *M. tuberculosis* culture filtrate antigens reacted with human sera. Abbreviations: NL, healthy control; TB, tuberculosis patient; LL, lepromatous leprosy patient; BB, borderline; BL, borderline lepromatous patient; BT, borderline tuberculoid patient. The 30- and 31-kDa antigens and the 32-kDa antigen are indicated on the left and right, respectively. Molecular weights are indicated on the left.

lepromatous and tuberculoid sera, these antigens may be useful for diagnostic purposes, for predicting the course of disease in a leprosy patient, and for understanding pathogenesis.

We thank Richard W. Truman, project officer of the National Hansen's Disease Serum Bank at the Gillis W. Long Hansen's Disease Center in Carville, La., and the World Health Organization IMMLEP Serum Bank for generously providing sera used in these studies. We also thank Rosalind Van Landingham and Laura Walker for providing reagents.

LITERATURE CITED

1. **Bothamley, G., J. Swanson Beck, I. Agusni, M. I. Ilias, T. Kardjito, J. M. Grange, and J. Ivanyi.** 1987. Antibodies to *Mycobacterium tuberculosis* in leprosy. Lancet **i**:1098.
2. **Britton, W. J., L. Hellqvist, R. J. Garsia, and A. Basten.** 1988. Antigens of *Mycobacterium leprae* identified by immunoprecipitation with sera from leprosy and tuberculosis patients. Clin. Exp. Immunol. **71**:394–398.
3. **Cohen, M. L., L. W. Mayer, H. S. Rumschlag, M. A. Yakrus, W. D. Jones, Jr., and R. C. Good.** 1987. Expression of proteins of *Mycobacterium tuberculosis* in *Escherichia coli* and potential of recombinant genes and proteins for development of diagnostic reagents. J. Clin. Microbiol. **25**:1176–1180.
4. **Daniel, T. M., R. C. Good, and B. W. Janicki.** 1975. Immunoelectrophoresis of *Mycobacterium tuberculosis* antigens. Am. Rev. Respir. Dis. **112**:639–644.
5. **Devries, R. R. P., L. E. Nijenhuis, R. F. M. L. A. Fat, and J. J. van Rood.** 1976. HLA-linked genetic control of host response to *Mycobacterium leprae*. Lancet **ii**:1328–1330.
6. **Gaylord, H., P. J. Brennan, D. B. Young, and T. M. Buchanan.** 1987. Most *Mycobacterium leprae* carbohydrate-reactive monoclonal antibodies are directed to lipoarabinomannan. Infect. Immun. **55**:2860–2863.
7. **Ivanyi, J., S. Sinha, R. Aston, D. Cussell, M. Keen and W. Sengupta.** 1983. Definition of species specific and cross-reactive antigenic determinants of *Mycobacterium leprae* using monoclonal antibodies. Clin. Exp. Immunol. **52**:528–536.
8. **Kenna, J. G., G. N. Major, and R. S. Williams.** 1985. Methods for reducing nonspecific antibody binding in enzyme-linked immunosorbent assays. J. Immunol. Methods **85**:409–419.
9. **Kim, S. J., I. H. Choi, S. Dahlberg, B. Nisperos, J. D. Kim, and J. A. Hansen.** 1987. HLA and leprosy in Koreans. Tissue Antigens **29**:146–153.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Myrvang, B., T. Godal, D. S. Ridley, S. S. Froeland, and Y. K. Song.** 1973. Immune responsiveness to *Mycobacterium leprae* and other mycobacterial antigens throughout the clinical and histopathological spectrum of leprosy. Clin. Exp. Immunol. **14**: 541–553.
12. **Olveira de Almeida, J. H. Brandao, and E. Garcia de Lima.** 1964. Enhanced serologic response of lepromatous patients to antityphoid vaccination. Int. J. Lepr. **32**:292–296.
13. **Pepys, J., R. Augustin, and A. B. Paterson.** 1959. Common antigenic components of mycobacterial extracts. Tubercle **40**: 163–172.
14. **Ridley, D. S.** 1974. Histological classification and the immunological spectrum of leprosy. Bull. W.H.O. **51**:451–465.
15. **Young, R. A., V. Mehra, D. Sweetser, T. Buchanan, J. Clark-Curtiss, R. W. Davies, and B. R. Bloom.** 1985. Genes for the major protein antigens of the leprosy parasite *Mycobacterium leprae*. Nature (London) **316**:450–453.