NEW BIOMEDICAL TECHNOLOGIES

Epitopic Mapping of Different Mycobacteria with the Use of Monoclonal Antibodies

L. N. Chernousova, V. I. Golyshevskaya, O. A. Kalinina, N. V. Kulikovskaya, M. A. Kapina, and V. I. Litvinov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 3, pp. 329-331, March, 1997
Original article submitted November 22, 1996

Monoclonal antibodies reacting with different mycobacteria are prepared. They are used for epitopic mapping of *Mycobacteria tuberculosis H37Rv, M. bovis bovinus 8, M. avium, M. kansasii,* and *M. smegmatis.* On the basis of the epitope maps 129 cultures of mycobacteria were identified by immunoblotting with the antibodies. The results of this identification coincided with those of microbiological investigation in 61.5-80% cases, depending on the genus of mycobacteria.

Key Words: *identification of Mycobacteria tuberculosis; monoclonal antibodies; antigenic determinant*

Classical identification of mycobacteria is based on analysis of their biological, cultural, and biochemical properties [9], which requires about 30 days. Mycobacteria have been identified by immunological and molecular-biological methods [3,6,8]. Immunological methods employing monoclonal antibodies (MAB) seem prospective.

Monoclonal antibodies to different antigenic determinants of mycobacteria were obtained at the Central Institute of Tuberculosis. In the present study we tested them for identification and epitope mapping of mycobacteria.

MATERIALS AND METHODS

Laboratory strains of mycobacteria were received from the museum of the Central Institute of Tuberculosis, Russian Academy of Medical Sciences. Wild strains from humans and animals were isolated at the Laboratory of Microbiology of the Central Institute of Tuberculosis, Institute of Experimental Veterinary Medicine, Institute of Veterinary Sanitation, and Czech Institute of Hygiene and Sanitation (Prague). Before immunization and selection of hybrid clones, *M. tuberculosis, M. bovis, M. avium, M. kansasii,* and *M. smegmatis* were irradiated, sonicated in a Bransonic 1510 disintegrator [4], and suspended in incomplete Freund's adjuvant.

This suspension was injected into BALB/c mice subcutaneously into 6 sites two times at a 2-week interval. Blood was analysed for specific antibodies 10-14 days after the last immunization. The resolving dose of the antigen (50 μg) was injected into the tail vein 3 days before isolation of splenocytes for hybridization. Monoclonal antibodies were prepared by the hybridoma technique using syngeneic NSO and P3×63Sp2/0 hybridomas [4].

Antibodies were selected by immunoenzyme assay with mycobacterial suspensions (10 μg/ml) as antigen. Antibodies to murine immunoglobulins of all classes were used as the second antibody. After sonication, mycobacterial suspension was electrophorezed in 12.6% SDS-polyacrylamide gel. Proteins with known molecular weight ranging from 92.5-14.4 kD (Pharmacia) served as markers.

Laboratory of Biotechnology, Central Institute of Tuberculosis, Russian Academy of Medical Sciences, Moscow

0007-4888/98/0003-0291$20.00

TABLE 1. Identification of Laboratory Strains of Mycobacteria by Expression of Epitope on Polypeptide of Certain Molecular Weight

Mycobacteria	Epitopes revealed by MAB (code and molecular weight, kD)					
	3C10	4C1	10D6	3H3	2H10	1D3
M. tuberculosis H37Rv			19-20	21		
M. bovis bovinus 8		16	19-20	21		
M. avium				21, 55		79
M. kansasii			22	21		
M. smegmatis	10			21	38	

Immunoblotting for determination of the molecular weight of polypeptides reacting with MAB included the following steps: 1) electrophoretic separation of sonicated mycobacteria in SDS-polyacrylamide gel, 2) electrophoretic transferring of protein from gel onto a nitrocellulose membrane, and 3) immunoenzyme assay with MAB on nitrocellulose. The calibration curve was constructed for determination of molecular weight of polypeptides bearing antigenic determinants reacting with the MAB [2,4].

RESULTS

We prepared MAB reacting in immunoenzyme assay with one (MABs 3C10, 4C1, 1D3, and 2H10) or several (MABs 10D6 and 3H3) genera of mycobacteria. Epitope "maps" of mycobacteria designated as 3C10, 4C1, 10D6, 3H3, 2H10, and 1D3 (according to MAB with which they reacted) are shown in Table 1. Our findings indicate that the 19-20-kD and 21-kD polypeptides from *M. tuberculosis* bear 10D6 and 3H3 epitopes, respectively. Expression of 4C1, 10D6, 3H3 epitopes on 16-, 19-20-, and 21-kD peptides, respectively, is typical of *M. bovis*. *Mycobacterium avium* is characterized by expression of 3H3 epitope on two polypeptides (21 and 55 kD) and by expression of 1D3 epitope on 79-kD polypeptide. In *M. kansasii* 10D6 epitope is carried by 22-kD polypeptide and 3H3 epitope by 21-kD polypeptide. *Mycobacterium smegmatis* is characterized by expression of 3C10 epitope on 10-kD polypeptide, 2H10 epitope on 38-kD polypeptide, and 3H3 epitope on 21-kD polypeptide.

We obtained 129 cultures of mycobacteria from pathological material of cattle and sputum of patients with tuberculosis. All these mycobacteria were identified by cultural and biochemical methods at the Central Institute of Tuberculosis by techniques described in Order No. 588 of June 8, 1978 of the Ministry of Health and NJH recommendations "Mycobacterial Culture Collection" (1980).

We attempted to identify these mycobacteria with MAB by immunoblotting using disintegrated microbial cells as an antigen. The results were compared with those of bacteriological investigation. Cultures producing intense band in the 19-20 kD area with MAB 10D6 and in the 21 kD area with MAB 3H3 were classified as *M. tuberculosis*. Cultures of *M. avium* produced bands in the 79 kD area with 1D3 MAB, in the 55 and 21 kD areas with 3H3 MAB. *Mycobacteria kansasii* were identified by bands in the 22 kD area with 10D6 MAB and in the 21 kD area with 3H3 MAB; *M. smegmatis* by bands in the 10 kD area with 3C10 MAB, in the 38 kD area with 2H10 MAB, and in the 21 kD area with 3H3 MAB; *M. bovis* by bands in the 19-20 kD area with 10D6 MAB, in the 16 kD area with 4C1 MAB, and in the 21 kD area with 3H3 MAB.

Using this approach, we identified 32 cultures as *M. tuberculosis* (out of 38 cultures identified by microbiological methods), i.e., 84.2%; 31 out of 40 cultures as *M. bovis* (77.5%); 16 out of 26 cultures as *M. avium* (61.5%); 16 cultures as *M. kansasii* (100 %); 8 out 9 cultures *M. smegmatis* (90%).

Attempts were made to identify *M. tuberculosis* and *M. avium* by an immunofluorescence ELISA using MAB reacting with mycobacteria of one genus [1,5,7].

In the present work for identification of mycobacteria we used MAB reacting with mycobacteria of several genera. The epitopes of these mycobacteria were mapped, and these maps were employed for identification of mycobacteria isolated from cattle and humans. The procedure with MAB is much shorter than microbiological investigation: it requires 3-5 days, which is important for timely diagnosis and correct choice of therapy.

REFERENCES

1. M. V. Androsova, M. A. Vladimirskii, and T. I. Alekseeva, *Probl. Tub.*, No. 5, 43-46 (1989).
2. N. V. Kulikovskaya, M. A. Kapina, O. A. Kalinina, and L. N. Chernousova, *Ibid.*, No. 1, 43-46 (1996).
3. V. I. Litvinov, *Ibid.*, 56-59.
4. L. N. Chernousova, N. V. Kulikovskaya, and V. S. Razumovskaya, *Ibid.*, No. 2, 49-51 (1990).
5. G. H. Bothamley, *Eur. Respir. J.*, **8**, No. 20, 676-688 (1995).

6. K. D. Eisenach, M. D. Gave, J. H. Bates, and J. T. Crawford, *J. Infect. Dis.*, **161**, 977-981 (1990).
7. A. H. J. Kolk, R. Evers, P. G. Groothuis, *et al. Infect. Immun.*, **57**, No. 8, 2514-2521 (1989).
8. R. J. Patel, J. W. H. Fries, W. F. Piessens, and D. F. Wirth. *J. Clin. Microbiol.*, **28**, No. 3, 513-518 (1990).
9. L. G. Wayne, in: *The Mycobacteria: a Source-Book*, G. P. Kubica, L. G. Wayne (Eds.), New York (1984), pp. 25-65.