Short Communication

Successful Preparations and Characterizations of Monoclonal Antibodies to a 65,000 Dalton Protein of *Mucobacterium leprae*

HIROKO NOMAGUCHI¹, MASANORI MATSUOKA², YASUYO MIYATA¹,
YUJI SHIGYO¹, KENJI KOHSAKA¹, SUBOWO³,
and TONETARO ITO¹

(Research Institute for Microbial Diseases, Osaka University, 8-1 Yamadaoka,
Suita, Osaka 561¹; National Institute for Leprosy Research, Higashimurayama,
Tokyo 189², Japan; Department of Histology, Faculty of Medicine,
Padjadjaran University, Bandung, Indonesia³.)

(Received for publication: September 26, 1988)

Key words: monoclonal antibody, epitope, 65kDa protein, mycobacteria, *Mycobacterium leprae*

Successful preparation and characterization of the monoclonal antibodies (MAbs) to *Mycobacterium leprae* antigens have been reported^(1,2,3), and some of them recognized the epitopes on the ≤ 65 kD protein of nonmycobacterial genera⁽⁴⁾. Four MAbs against 65kD protein which were presumed to recognize different epitopes on mycobacteria from reported ones⁽²⁾ are newly isolated in our laboratory. Here, we report on the four MAbs, and the new additional data on the MA-3A which was the common monoclonal antibody against 65kD protein of mycobacteria species as previously reported⁽³⁾.

The procedures for the antigen preparation and production of MAbs were described in detail previously⁽³⁾. Briefly, *M. leprae* and other species of mycobacteria were sonicated, and adjusted to a protein concentration of 1mg/ml and is referred to as 'sonicate antigen'. The preparation of murine MAb to *M. leprae* were performed from BALB/c -P3X63-Ag 8.653 fusion in the presence of polyethylene glycol 4000. Hybridomas were tested for antibody production by ELISA.

By initial screening by ELISA, 41 wells out of 288 seeded wells showed positive results of antibody production to *M. leprae* sonicate antigen. Each of the 41 culture supernatants was reacted in a Western blot against *M. leprae* sonicate antigen, and all of these culture supernatants were bound to the band of 65kD protein. For secondary screening, a dot blotting analysis was implicated to examine the cross-reactivity of these 41 culture supernatants against four mycobacterium bacilli, *M. leprae*, *M. scrofulaceum*, *M. marinum*, *M. lepraemurium*, which were spotted onto nitrocellulose strips. These 41 culture supernatants were grouped into six groups in accordance with their species specificity. Six hybridomas were selected from each group, and cloned using limiting dilution technique. Six cloned cells were injected into the peritoneal cavity of BALB/cAjl mice primed with pristane to produce antibody rich ascites. Only four clones of MAbs (A2A91, A5B-16, A2D-1, B2C-18) were rich antibody producer in mouse ascites, and other two clones were poor producer.

Fig. 1 Western blot results with MABs. For each lane, the sonicate antigen of *M. leprae* was electrophoresed on 12.5% SDS PAGE and transferred to nitrocellulose. Lane 1 was reacted with MAB A2D-1; lane 2, MAB A2A-91; lane 3, MAB A5B-16; lane 4, MAB B2C-18; lane 5, MAB 3A; lane 6, MAB IIIIE9.

Fig. 2 Western blot results with MAB 3A. For each lane, the sonicate antigen of bacilli was electrophoresed on 12.5% SDS-PAGE and transferred to nitrocellulose. Lane 1, JM83/pUC-N5 (overproduced strain for 65K); lane 2, JM83/pUC8 (vector control); lane 3, *Acinetobacter calcoaceticus* (ATCC 23055); lane 4, *Corinebacterium diphtheriae* (C7(-)); lane 5, *Haemophilus influenzae* B (ATCC 9795); lane 6, *Nocardia asteroides*; lane 7, *Nocardia nova*; lane 8, *Pseudomonas aeruginosa* (ATCC 23268); lane 9, *Propionibacterium acnes*; lane 10, *Streptococcus pneumoniae* (ATCC 11733); lane 11, *M. leprae*.

Each of the four clones which was rich MABs producer was reacted in a Western blot against sonicate *M. leprae* solution. As a control, two other established MABs, 3A which recognized common epitopes on 65kD-protein of mycobacteria⁽³⁾, and IIIIE9 which was generated in Dr. Buchanan's Laboratory as *M. leprae* MAB⁽⁵⁾, were reacted against the same antigens in a Western blot. As shown as Fig. 1, all six MABs recognized bands of ≤ 65 kDa in the sonicate antigen of *M. leprae*.

The dot blot immunoassay studies of species specificity for the four different MABs are shown in Table 1. The dot reactions were graded as negative or positive in an intensity range of 1 (low) to 4 (high). The MABs recognized epitopes found on as few as 3 (A2D-1) to as many as 14 (B2C-18) of the 21 species of mycobacteria tested. By Buchanan et al's report, fourteen distinct epitopes have been recognized by a group of 23 MABs directed at the 65-kD molecule of *M. leprae*⁽⁵⁾. The sequences of the epitopes recognized by MAB IIIIE9, IIC8 and IIC8 have been confirmed with solid-phase peptide synthesis by Brown *et al.*⁽⁶⁾ and Anderson *et al.*⁽⁷⁾ beginning with sequence information provided by Mehra *et al.*⁽⁸⁾. The species specific patterns by newly isolated 4 MABs in our laboratory were different from the patterns of other 14 MABs reported by Buchanan *et al.*⁽⁵⁾. These four MABs maybe recognize the different epitopes from 14 epitopes on the 65-kD protein of *M. leprae* reported. It is important what epitopes are recognized by these 4 MABs, however it remains to be elucidated. We are going to see the sequence of

Table 1 Species specificity in dot blot immunoassays of MAbs which recognize epitopes on the 65-kDa protein of *M. leprae*

Mycobacterial species	Reactivity of MAb ^a					
	A2D-1	A2A-91	A5B-16	B2C-18	3A	III E9
<i>M. avium</i>	—	—	—	+	++	—
<i>M. bovis</i> BCG	—	+	+	###	###	—
<i>M. chelonae</i>	+	###	+	—	###	—
<i>M. chitae</i>	—	++	+	—	++	—
<i>M. fortuitum</i>	—	—	—	—	++	—
<i>M. gastri</i>	—	—	—	—	+	—
<i>M. goodii</i>	—	—	—	+	+	—
<i>M. intracellulare</i>	—	—	—	+	++	—
<i>M. kansasii</i>	—	—	—	+	++	—
<i>M. leprae</i>	###	###	++	###	###	###
<i>M. marinum</i>	++	###	###	###	###	—
<i>M. nonchromogenicum</i>	—	—	—	—	+	—
<i>M. scrofulaceum</i>	—	++	+	###	++	—
<i>M. smegmatis</i>	—	+	—	—	+	—
<i>M. szulgai</i>	—	—	—	+	+	—
<i>M. terrae</i>	—	++	+	###	++	—
<i>M. triviale</i>	—	—	+	+	+	—
<i>M. tuberculosis</i> H37Ra	+	+	+	++	++	—
<i>M. tuberculosis</i> H37Rv	+	###	++	###	###	—
<i>M. tuberculosis</i> Aoyama	—	+	++	++	++	—
<i>M. lepraemurium</i> Ogawa 7th	—	++	++	++	++	—

^a Reactions were graded as negative (—) or from + to ### in order of increasing intensity of the dot reaction.

the epitopes.

We described in the previous paper that the MAb-3A recognized all of the epitopes on 65kD-protein of mycobacteria tested (3). In our recent study, in addition to the results, the MAb-3A was determined as the monoclonal antibody that recognized also the epitopes on 65kD-protein of nonmycobacterial genera tested as shown as in Fig. 2. in Western blotting assay. The MAb 3A did not recognize the epitopes on mammalian cells (tissue culture cells; 20=Fisher rat skin which lacks thymidine kinase, L=mouse, syn=human neuroblastoma, TC98=Lewis rat schwannoma, MK-1=macrophage, A31=mouse 3T3).

These antibodies should be useful reagents for analysis of epitopes which stimulate T cells, and for purification of 65-kD protein by affinity chromatography, and identification of mycobacterium. In our recent study, MAb-3A was used for the detection of 65-kD protein of *M. leprae* expressed in *E. coli* and purification of that protein with affinity chromatography.

We thank Professor Atsuo Nakata for his kind advice.

We are grateful to the Laboratory for Culture collections, Research Institute for Microbial Diseases-Osaka University and Mr. M. Higashitsutsumi, Research Foundation for Microbial

Diseases-Osaka University for providing the nonmycobacterial species in this study, and the WHO IMMLEP monoclonal antibody bank for supplying MAb IIIIE9.

This study was supported by the grant of the Sasakawa Memorial Health Foundation and scholarship of the Taniguchi Memorial Fellowship for Drs. Matsuoka and Subowo.

Reference

- 1) Engers, H. D., Houba, V., Bennedsen, J., Buchanan, T. M., Chaparas, S. D., Kadival, G., Closs, O., David, J. R., Embden, van J. D. A., Godal, T., Mustafa, S. A., Ivanyi, J., Young, D. B., Kaufmann, S. H. E., Khomenko, A. G., Kolk, A. H. J., Kubin, M., Louis, J. A., Minden, P., Shinnick, T. M., Trnka, L. and Young R. A.: Results of a World Health Organization-sponsored workshop to characterize antigens recognized by mycobacteria-specific monoclonal antibodies. *Infect. Immun.*, **51** : 718-720, (1986).
- 2) Engers, H. D., Abe, M., Bloom, B. R., Mehra, V., Britton, W., Buchanan, T. M., Khanolkar, S. K., Young, D. B., Closs, O., Gillis, T., Harboe, M., Ivanyi, J., Kolk, A. H., and Shepard, C. C.: Results of a World Health Organization-sponsored workshop on monoclonal antibodies to *Mycobacterium leprae*. *Infect. Immun.*, **48** : 603-605, (1985).
- 3) Matsuoka, M., Nomaguchi, H., Miyata, Y. and Ito, T.: Production of monoclonal antibody to a 65,000 dalton protein of *Mycobacterium leprae*. *Jap. J. Lepr.*, **56** : 21-25, (1987).
- 4) Young, D. B., Ivanyi, J., Cox, J. H. and Lamb, J. R.: The 65kDa antigens of mycobacteria-a common bacterial protein? *Immunol. Today*, **8** : 215-219, (1987).
- 5) Buchanan, T. M., Nomaguchi, H., Anderson, D. C., Young, R. A., Gillis, T. P., Britton, W. J., Ivanyi, J., Kolk, A. H. J., Closs, O., Bloom, B. R. and Mehra, V.: Characterization of antibody-reactive epitopes on the 65-kilodarton protein of *Mycobacterium leprae*. *Infect. Immun.*, **55** : 1000-1003, (1987).
- 6) Brown, W. B., Larrabee, W. A. and Kim, P. S.: Analysis of a leprosy-specific antibody epitope. *Lepr. Rev.*, **57** : 157-162, (1986).
- 7) Anderson, D. C., Young R. A. and Buchanan T. M.: Solid phase peptide synthesis of epitope that react with monoclonal antibodies to the 65,000 dalton protein of *Mycobacterium leprae*. *Lepr. Rev.*, **57** : 169-175, (1986).
- 8) Mehra, V., Sweetser, D. and Young, R. A.: Efficient mapping of protein antigenic determinants. *Proc. Natl. Acad. Sci. USA*, **83** : 7013-7017, (1986).

らい菌のダルトン蛋白質に対するモノクローナル 抗体の分離と性質

野間口博子¹ 松岡正典¹ 宮田泰代¹
執行祐爾¹ 高坂健二¹ スボオ³
伊藤利根太郎¹

(大阪大学微生物病研究所¹, 国立多摩研究所², パジャジャラン大学³)

らい菌菌体成分に対するモノクローナル抗体 3A (MAb 3A) について既に報告した。今回は新たに分離した抗体について、また MAb 3A についての新しい知見について報告したい。

前回とほぼ同様の方法を用いてモノクローナル抗体を 4 クローン分離した。これらは全て菌体 65kD に対する抗体であった。また、これら 4 種類の MAb の他の抗

酸菌との交差反応を Dot blotting を用いて検討したところ、らい菌特異抗体ではなく検査した 20 種類の抗酸菌のうち、5 種類の抗酸菌のエピトープを認識するものから 15 種類のを認識するものまで多種であった。

MAb 3A は、検査した全ての抗酸菌と交差したが、これに加えて大腸菌など抗酸菌ではない菌体の $\leq 65\text{kD}$ 蛋白質をも認識した。