Symposium on the IMMUNOLOGY OF LEPROSY, Oslo, Norway, 1986
Lepr Rev (1986) 57, Suppl 2, 63-66

Characterization of mycobacterial species specificity of 14 separate epitopes which reacted with monoclonal antibodies to the 65,000 molecular weight protein molecule of *Mycobacterium leprae*

T M BUCHANAN, H NOMAGUCHI, D C ANDERSON, R A YOUNG, T P GILLIS, W J BRITTON, J IVANYI, A H J KOLK, O CLOSS, R BLOOM & V MEHRA
University of Washington, Seattle, WA, Whitehead Institute for Biomedical Research, Cambridge, MA, Gillis W. Long Hansens Disease Center, Carville, LA, Albert Einstein College of Medicine, Bronx, Ny, USA, Osaka University, Osaka, Japan, University of Sydney, Sydney, Australia, Tuberculosis Unit, Hammersmith Hospital, London, England, Royal Tropical Institute, Amsterdam, The Netherlands, and National Institute of Public Health, Oslo, Norway

This study examined the number of different epitopes recognized by 23 monoclonal antibodies known to recognize the 65K dalton protein of the leprosy bacillus, and the mycobacterial species specificity of those 14 monoclonal antibodies shown to recognize different epitopes.

Materials and methods

MONOCLONAL ANTIBODIES (MOAb)

All of the monoclonal antibodies studied recognized the 65K dalton protein band of *M. leprae* as identified by Western Blot (1) or Gel-Immuno-Radio-Assay (2). Antibodies IIC8, IIIC8, IIIE9, IVD8, IIH9, and IVD2 were obtained from Dr. T.M. Buchanan, Seattle, Washington (3-5), antibodies Y1.2, E3.14, T2.3 and C1.3 from Drs. Vijay Mehra and B.R. Bloom, Bronx, New York (3), antibodies SL12, SL14, SL22, SL26, and SL28 from Dr. W.J. Britton, Sydney, Australia (6), antibody ML-30 from Dr. J. Ivanyi, London, England (7), antibody E423 from T. Gillis, Carville, Louisiana (5), antibodies F47-10, F67-2, F67-13, and F67-18 from Dr. Arend H.J. Kolk, Amsterdam, The Netherlands, and antibodies MOC2 and MOC6 from Dr. O. Closs, Oslo, Norway.

Each monoclonal antibody was purified by staphylococcal protein A or sizing chromatography and the purified antibody was radiolabelled with ^{125}I using a chloramine T (8) or Bolton-Hunter (8) procedure. An antigenic preparation of sonicated *M. leprae* (9) was coated to Immulon I removawells (Dynatech). The binding of each ^{125}I-labelled antibody to the antigen coated wells was evaluated for inhibition by a 1:100 dilution of ascites fluid of each monoclonal antibody. Monoclonal antibodies with different inhibition patterns from all of the other MOAbs were presumed to recognize different epitopes, and when more than a sin-

gle antibody recognized a given epitope, a single representative antibody was chosen for further study. In some instances where the radiolabelled antibody reacted poorly with antigen bound to polystyrene plates, the antibody was reacted with the same antigen bound to nitrocellulose. Inhibition of this binding by unlabelled MOAb in a 1:100 dilution was evaluated by autoradiography.

EVALUATION OF MYCOBACTERIAL SPECIES SPECIFICITY

Bacterial sonicate antigens of each of 23 mycobacterial species were prepared as previously described (9) and adjusted to a l mg/ml protein concentration. The species tested were *M. bovis* BCG, *M. bovis, M. chelonei, M. diernhoferi, M. duvali, M. flavescens, M. fortuitum, M. gastri, M. gordonae, M. intracellulare, M. kansasii, M. leprae, M. marinum, M. nonchromogenicum, M. peregrinum, M. phlei, M. scrofulaceum, M. smegmatis, M. terrae, M. thamnopheos, M. triviale, M. tuberculosis* H37Rv, *M. ulcerans,* and *M. vaccae.* One microgram protein in five microliters volume was spotted onto nitrocellulose in a template of the 23 species plus a buffer negative control per paper strip and allowed to air dry. The nitrocellulose template strips were blocked at 37°C for 1 hour with 5 % nonfat milk in phosphate buffered saline (PBS), and then incubated with a 1:500 – 1:2000 dilution of ascites fluid containing each respective monoclonal antibody in PBS containing 1 % nonfat milk, at room temperature (RT) for 30 minutes. The template strips were drained of antibody and washed x 5 minutes in PBS with gentle shaking at RT, followed by washing x 15 minutes in 0.5M NaCI in phosphate buffer containing. 05 % Tween-2Ø, followed by a second 5 minute wash with PBS. The goat anti-mouse immunoglobulin peroxidase conjugate (Cappel) was diluted 1:2000 in 1 % nonfat milk-PBS and incubated with the template strips at RT for 30 minutes. The strips were then drained, and washed at RT with PBS x1 x 5 min, followed by 4 washes of 10 minutes each in 0.5M NaCI in PBS with .05 % Tween 20, followed by a final wash in PBS x 5 minutes. The washed template strips were reacted for 30-45 minutes with a .015 % H_2O_2 – color development (4-Cl-l-naphthol, BioRad) solution until the dots developed a purple color. The templates were then washed with distilled water several times and air dried and stored in the dark or in some cases were stored in distilled water prior to air drying and storage. The dot reactions were graded as negative (-) or positive in a range of 1 (low) to 4 (high) intensity.

Results

Of the 23 MOAbs studied, 14 appeared to recognize different epitopes based upon cross-competition studies. The distinct MOAbs and other MOAbs which had identical reactivities to those 14 are summarized in Table 1.

The species specificity of the 14 distinct epitopes recognized is summarized in Table 2. A group of 5 MOAbs represented by IIIE9 recognized an epitope found only on *M. leprae,* and another antibody (F67-13) recognized all sonicate antigens weakly ($\leqslant$ 1) but gave a 2+ reaction with *M. leprae.* MOAb ML-30 reacted strongly with only 3 species and the remaining MOAbs reacted with eight or more species. Only MOAb IIC8 reacted with all 23 mycobacterial species tested.

Acknowledgements

The authors are grateful to the WHO IMMLEP monoclonal antibody bank for providing some of the antibodies used in this study, and to Duane Olsen, Nancy Jenkins, Charlotte Richards and Sigrid McAlister for excellent technical assistance. This study was supported in part by the IMMLEP component of the UNDF/World Bank/WHO Special Program for Research and Training in Tropical Disease, by the Rockefeller foundation Program for Re-

search on Great Neglected Diseases, by Public health Service Grant AI-22007 and Contract NO1 AI-52582 from the National Institute of Allergy and Infectious Disease, National Institutes of Health, and by a grant from the Japanese component of the US-Japan Collaborative Program in Leprosy for Dr. Nomaguchi.

Table 1. MOAbs which appeared distinct as evaluated by cross-competition studies

MOAb(s)	*MOAbs other than homologous which inhibited*
IIC8	F67-2 (69 %)
F67-2	IIC8 (95 %)
IIIC8	none
ML-30	none
Y1.2	none
IIIE9, IVD8, MOC2, SL12, SL26	IVD2 (67 %) and each of the group of 5 *M. leprae* specific MOAbs listed which all recognized the same epitope
IVD2	IIIE9 (51 %)
C1.3	E423 (24 %)
IIH9, E3.14, MOC6	IVD2 (22 %)
E423	none
SL22	ML-30 (20 %)
SL28	none
F67-13	none
F67-18	none

Table 2. Species specificity of 14 distinct epitopes recognized by MOAbs on the 65,000 dalton protein of* M. leprae

Epitope (MOabs)	*Mycobacterial Species** Recognized*
IIC8	all species
F67-2	bov GCG, bov, chel, flav, gas, gord, kan,lep, phlei, scrof, smeg, ter, tham, tub, and ulc.
IIIC8	dier, duv, flav, gas, gor, lep, per, scrof, smeg, ter, tham, ulc and vac.
ML-30	lep, tham, and tub.
Y1.2	bov BOG, chel, duv, flav, gas, gord, int, kan, lep, scrof, ter, tham, tub, and ulc.
IIIE9, IVD8, MOC2, SL12, SL26	lep
IVD2	bov, chel, duv, flav, gas, gor, lep, scrof, ter, tham, and ulc.
C1.3	bov, flav, gord, kan, lep, scrof, tham, and ulc.
IIH9, E3.14, MOC6	bov BCG, chel, flav, gor, kans, lep, scrof, ter, tham, tub, and ulc.
E423	bov BCG, bov, duv, flav, gas, gor, int, kan, lep, per, scrof, smeg, ter, tub, and ulc.
SL22	bov BCG, bov, chel, duv, flav, gas, gor, int, kan, lep, phlei, scrof, smeg, ter, tham, tub, ulc, and vac.

Epitope (MOabs)	*Mycobacterial Species** Recognized*
SL28	bov BCG, chel, dier, duv, flav, fort, gas, gor, int, kan, lep, mar, non, per, ter, tham, triv, tub, and ulc.
F67-13	lep
F67-18	bov BCG, bov, chel, duv, flav, gas, gor, int, kan, lep, per, phlei, scrof, smeg, ter, tham, triv, tub, ulc and vac.

* = The sonicate antigen preparation from these species of mycobacteria produced a dot reaction of 2-4 intensity (scale 0-4) in dot blot immunoassays with the MOAb indicated.

** bov BCG = *M. bovis* BCG, bov = *M. bovis,* chel = *M. chelonei,* dier = *M. diernhoferi,* duv = M. duvali, flav = *M. flavescens,* fort = *M. fortuitum,* gas = *M. gastri,* gor = *M. gordonae,* int = *M. intracellulare,* kan = *M. kansasii,* lep = *M. leprae,* mar = *M. marinum,* non = *M. nonchromogenicum,* per = *M. perigrinum,* phlei = *M. phlei,* scrof = *M. scrofulaceum,* smeg = *M. smegmatis,* ter = *M. terrae,* tham = *M. thamnopheos,* triv = *M. triviale,* tub = *tuberculosis,* ulc = *M. ulcerans,* vac = *M. vaccae*

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