Letter to the Editor

Results of a World Health Organization-Sponsored Workshop to Characterize Antigens Recognized by Mycobacterium-Specific Monoclonal Antibodies

An international workshop organized and sponsored by the Immunology of Tuberculosis (IMMTUB) component of the World Health Organization (W.H.O.) Vaccine Development Programme to characterize the specificity and reaction patterns of murine monoclonal antibodies (MAbs) raised against various mycobacteria was held in Geneva, 3 to 5 June 1985. A total of 31 MAbs (28 ascites and 3 culture supernatants) generated in nine different laboratories using several mycobacterial antigens for immunization (*Mycobacterium tuberculosis*, virulent and avirulent strains, *M. bovis* BCG, and *M. leprae*) were submitted in early 1985 to the IMMTUB MAb bank located in the W.H.O. Immunology Research and Training Center (W.H.O./I.R.T.C.) in the Department of Pathology, University of Geneva. The samples were coded, aliquoted, and distributed to 12 laboratories for independent analysis by a variety of methods. Eight laboratories tested the reactivity and specificity of the MAbs using a spectrum of antigens prepared from up to 23 species of mycobacteria. The assay methods included enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), indirect immunofluorescence assay (IFA) and several additional related techniques, e.g., dot blots, Western blots, sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoperoxidase technique (SGIP), gel immunoradioassay (GIRA), etc. Several laboratories used the same MAbs as probes to screen mycobacterium-derived recombinant DNA libraries for the expression of mycobacterium-specific protein antigens.

In addition to the MAbs, several laboratories submitted various mycobacterial antigen preparations for testing. These antigens were derived by three different approaches: (i) preparations eluted from MAb immunoadsorbant columns, (ii) an antigen preparation (nonpurified) obtained by recombinant DNA techniques and expressed in *Escherichia coli*, and (iii) a synthetic peptide prepared by conventional solid-state peptide synthesis methods.

All of the tests were performed blindly on coded samples by the participating laboratories, and the results were sent to the W.H.O/I.R.T.C. in Geneva for compilation. The code was broken during the workshop discussion, and the results were analyzed and compared with regard to the following criteria: (i) reactivity and specificity of MAbs for mycobacterial antigens, (ii) determination of the molecular size(s) and protease susceptibility of the antigen molecules, (iii) ability of the MAbs to detect phage in an *M. tuberculosis* λgt11 recombinant DNA library which expressed mycobacterium-specific target antigens, and (iv) stimulation of murine or human mycobacterium-specific T-cell populations and clones to proliferate and secrete lymphokines in vitro.

The findings of individual investigators were discussed in detail during the workshop, and the results agreed upon by the participants are summarized in Tables 1 and 2.

Table 1 lists the MAbs distributed, the results obtained for mycobacterial specificities, and the molecular size(s) and structure(s) of the antigens recognized by the MAbs. The specificity patterns of the MAbs for different mycobacteria varied and for the purpose of this report were divided into four categories: *M. tuberculosis* specific; *M. tuberculosis* complex (i.e., reacting with *M. tuberculosis*, *M. bovis* BCG, and *M. africanum*); limited cross-reactivity (reacting with a restricted number of additional mycobacteria), and broad cross-reactivity (reacting with a majority of the strains tested). The results for ELISA and RIA were similar for the vast majority of MAbs tested; hence, they were grouped together (with one exception, IT 21). There was substantial variation in the apparent molecular sizes of the antigens recognized by the MAbs (ranging from 12 to 80 kilodaltons [kDa], with single to multiple bands). However, several MAbs reacted with molecules of similar (if not identical) size, and in many cases the reaction pattern was related to the mycobacterial specificity, as summarized below. (i) None of the MAbs was found to be specific for a given mycobacterial species or strain when the results of all of the tests were taken into account. (ii) Six of the MAbs tested showed a specificity restricted to *M. tuberculosis* complex. They could be divided into two groups according to their reactivity patterns with two different molecular sizes: three MAbs (IT 1, 4, and 20) reacted with a 14-kDa molecule and another three (IT 15, 21, and 23) reacted with a 38-kDa molecule. (iii) The limited cross-reactivity pattern was represented by a series of MAbs which recognized a wide spectrum of molecular sizes: 12-kDa (IT 3), 71-kDa (IT 11), 19-kDa (IT 10, 12, 16, and 19), and multiband 65-kDa (IT 13, 31, and 33) antigens. (iv) Of the broadly cross-reactive MAbs, two (IT 17 and 29) reacted with a 23-kDa molecule, another two (IT 9 and 32) showed multiband reactivity (20 to 80 kDa), and several others could not be classified due to poor reactions in the assay systems used.

The antigens recognized by the MAbs were identified as proteins based on their sensitivity to treatment with proteolytic enzymes such as subtilisin. In several cases, however, the actual nature of the antigen could not be established.

There was no apparent relationship between the antigens and the immunization schedules used for production of the MAbs on the one hand and the characteristics of the MAbs as judged by their specificity and reaction patterns (Table 1) on the other.

The MAbs were used as probes by two laboratories to detect phage in an *M. tuberculosis* λgt11 recombinant DNA library which expressed target antigens recognized by the MAbs. Initially, a pool of the 33 MAbs was used to screen approximately 10^6 recombinant phage, and 134 positive signals were detected. Twenty-eight additional positive signals were obtained when a screening was carried out using a pool consisting of antibodies IT 1, 4, 13, 20, 21, 23, and 27. The phage corresponding to the positive signals were plaque purified and tested for reactivity with the individual MAbs to identify which target antigen was expressed. Six main groups of reactivity patterns were observed (Table 2). In one group (reactivity patterns A, B, C, and D), 22 phage reacted with MAbs IT 1, 4, and 20, 2 phages reacted with IT 1 and 4, 1 phage reacted with IT 4 and 20, and 1 phage reacted with IT 20 only. These data suggest that MAbs 1, 4, and 20 react with the same target antigen but not with the same epitopes on that antigen. This interpretation is consistent with cross-competition binding data obtained using radiolabeled MAbs. Similarly, the reactivity patterns of a second group of phage (35 phage in patterns E, F, G, H, and I) indicate that antibodies IT 13, 31, and 33 react with the same antigen but

TABLE 1. Characterization of MAbs to mycobacteria submitted to the IMMTUB MAb bank

MAb[a]		Immuno-globulin subclass	Mycobacterial specificity[b]				Molecular size (kDa)[c]	Nature of antigen[d]	Comments	Reference
IT no.	Designation		ELISA-RIA	Dot blot	IFA	Western SGIP				
1	F 23-49	IgG2a	Mt-C	Mt-C	Mt-C	Mt-C	14*	Protein		3, 4
2	25 D 4		CR-B	CR-B	Neg.	Neg.	Unknown	Unknown	Weak activity	
3	SA 12	IgG2a	CR-L	CR-L	CR-L	CR-L	12	Protein		5
4	F 24-2	IgM	Mt-C	Mt-C	Mr-C	Mt-C	14*	Protein		3, 4
5	25 E 3		CR-B	CR-B	CR-B	CR-B	Unknown	Unknown	Nonspecific binding	
6	MT 107 A 4/5	IgM	CR-B	CR-B	CR-B	CR-B	Unknown	Unknown	Nonspecific binding	
7	F 29-29	IgGa2	Weak	CR-B	Weak	CR-L	40	Protein		3, 4
8	18 B 10		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.		
9	MT 107 F5/C5	IgM	CR-B	CR-B	CR-B	CR-B	20–80	Protein (?)	Multiple bands	
10	F 29-47	IgM	CR-L	CR-L	CR-L	CR-L	19†	Protein		3, 4
11	51 A	IgG2a	CR-L	CR-L	Mt-C	CR-L	71	Protein		
12	HY T 6	IgG	CR-L	CR-L	CR-L	CR-L	19†	Protein		C. Shou et al.[e]
13	WTB 78-A1	IgG1	CR-L	CR-L	CR-L	CR-L	65‡	Protein	Multiple bands	1
14	TB-C-14	IgG2a	Weak	CR-B	CR-L	Neg.	Unknown	Unknown	Weak activity	
15	WTB 72-A6	IgG1	CR-L	Mt-C	Neg.	Mt-C	38§	Protein		1
16	66	IgG2a	CR-L	CR-L	CR-L	CR-L	19†	Protein		
17	SA1D2D1	IgG1	CR-B	CR-B	CR-B	CR-B	23	Protein		D. B. Young et al.[f]
18	TB-C-15	IgG2a	Neg.	Neg.	Neg.	Neg.	Unknown	Unknown		
19	WTB 23-A1	IgG1	CR-L	CR-L	CR-L	CR-L	19†	Protein		
20	WTB 68-A2	IgG1	Mt-C	Mt-C	Neg.	Mt-C	14*	Protein	Similar epitope to IT 1	1
21	HY T 28	IgG	Mt-C	Mt-C	Neg.	Mt-C	38§	Protein	Weak ELISA, strong RIA	
22	TB-C-15	IgG2a	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.		
23	WTB 71-A2	IgG2b	Mt-C	Mt-C	Neg.	Mt-C	38§	Protein	Different epitope than IT 15 and 21	1
24	TB-C-14	IgG2a	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.		
25	TB-C-14	IgG2a	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.		
26	TB-C-15	IgG2a	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.		
27	TB-C-13	IgG2a	Weak	Mt-C	Neg.	Weak	19	Protein	Different epitope than IT 10, 12, 16, 19	
28	TBI B6 F4	IgM	Neg.	Neg.	Neg.	Neg.	Unknown	Unknown		
29	TBI B2 H2	IgG3	Weak	CR-B	Neg.	Neg.	23	Protein (?)		
30	TBI A II C3	IgA	Weak	Mt-a	Neg.	Neg.	Unknown	Unknown	To be confirmed	
31	SA2 D5 H 4	?	CR-L	CR-L	Neg.	CR-L	65‡	Protein	Multiple bands	
32	SA2 A1 A5	IgM	CR-B	CR-8	CR-N	Weak	20–80	Unknown	Nonspecific binding	
33	ML II H 9	IgG1	CR-L	CR-L	CR-L	CR-L	65‡	Protein		2

[a] The IT number represents the IMMTUB reference number. The designation is that provided by the suppliers. The MAbs were contributed by the following investigators (IT numbers): J. Bennedsen, 12 and 21; T. M. Buchanan, 17, 28, 29, 30, 31, 32, and 33; O. Closs, 11 and 16; T. M. Daniel, 14, 18, 22, 24, 25, 26, and 27 (samples 14 and 25 were aliquots of the same MAb, as were samples 18 and 26); J. Ivanyi, 13, 15, 19, 20, and 23; A. G. Khomenko, 2, 5, and 8; A. H. J. Kolk, 1, 4, 7, and 10; M. Kubin, 6 and 9; P. Minden, 3. All of the MAbs were provided as ascites except IT 22, 24, and 27, which were culture supernatants precipitated by ammonium sulfate. Some of these MAbs have been partially described in previous publications (see relevant references).

[b] Mycobacterial specificity on antigens prepared from up to 23 species of mycobacteria was tested by ELISA, RIA, dot blots (D. B. Young, M. J. Fohn, S. R. Khanolkar, and T. M. Buchanan, Clin. Exp. Immunol., in press), IFA, Western blots (Young et al., in press), and SGIP (3). Categories of specificity: Mt-C, *M. tuberculosis* complex (i.e., reacting with *M. tuberculosis* virulent or avirulent strains, *M. bovis* BCG, and *M. africanum*); CR-L, limited cross-reactivity (i.e., reacting with a restricted number of other mycobacteria); CR-B, broad cross-reactivity (i.e., reacting with a majority of the strains tested); Mt-a, specific for *M. tuberculosis* avirulent strain (IT 30); Weak, weak reactivity; Neg., negative.

[c] Molecular sizes were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with different immunoblotting techniques (2–5; C. Schou, Z. L. Yuan, J. Bennedson, and A. B. Andersen, submitted for publication; Young et al., in press) and expressed in kDa. Symbols: *, antigen recognized by MAbs IT 1, 4, and 20; †, antigen recognized by IT 10, 12, 16, and 19; ‡, antigen recognized by IT 13, 31, and 33; §, antigen recognized by IT 15, 21, and 23 (IT 23 recognized an epitope different than that recognized by IT 15 and 21).

[d] Antigens were classified as protein in nature if they were sensitive to the proteolytic enzyme subtilisin.

[e] Shou et al., submitted.

[f] Young et al., in press.

not the same epitopes. Seventy-eight phage reacted with MAb 11 (pattern J), and two phage reacted with MAb 27 (pattern K). One phage reacted with all of the MAbs tested. This phage may be expressing an immunoglobulin-binding protein rather than an actual mycobacterium-derived MAb target antigen. Finally, 20 of the 162 phage tested did not react with any of the individual or pooled MAbs in this assay system. In summary, the recombinant DNA results were in good agreement with those presented in Table 1. Thus, the MAb analysis of recombinant DNA clones should allow further studies to be undertaken to characterize the genes.

Several mycobacterial antigen preparations were tested for their ability to stimulate various mycobacterium-specific human and murine T-cell populations and clones in vitro. Although no definitive results were obtained, several general conclusions can be drawn from these experiments. (i) Certain preparations which had been purified by elution from MAb immunoadsorbant columns appeared to contain several antigenic determinants (molecules). (ii) Mycobacterial antigens expressed in *E. coli* will have to be purified from the contaminating bacterial proteins before testing their ability to stimulate heterogeneous T-cell populations in vitro. (iii)

TABLE 2. Dissection of mycobacterial tuberculosis antigens using recombinant DNA probes and MAbs[a]

Reaction pattern	Reaction with IMMTUB MAbs								No. of phage clones
	1	4	20	13	31	33	11	27	
A	−	−	+	−	−	−	−	−	1
B	+	−	+	−	−	−	−	−	2
C	−	+	+	−	−	−	−	−	1
D	+	+	+	−	−	−	−	−	22
E	−	−	−	+	ND	ND	−	−	4
F	−	−	−	+	−	+	−	−	1
G	−	−	−	+	+	+	−	−	25
H	−	−	−	−	+	+	−	−	3
I	−	−	−	−	−	+	−	−	2
J	−	−	−	−	−	−	+	−	78
K	−	−	−	−	−	−	−	+	2
L	+	+	+	+	+	+	+	+	1
M	−	−	−	−	−	−	−	−	20

[a] The IMMTUB MAbs listed are among those described in Table 1. The remaining 24 MAbs did not react with any of the individual phage tested in this assay system. The assays were performed as described in reference 6, using the same recombinant DNA library. ND, Not determined.

The low-molecular-weight synthetic peptide (12 amino acids) did not react in any of the assay systems utilized. Such peptides will probably require polymerization or coupling to a carrier molecule to be effective as immunogens or antigens.

The use of these MAbs together with purified mycobacterial antigens should allow the following questions to be investigated. What is the peptide structure of epitopes recognized by MAbs and T-cell clones? What is the nature of the cell-mediated response(s) elicited in *M. bovis* BCG-vaccinated individuals or tuberculosis patients tested using the purified antigens? These experiments should be conducted (i) in vitro, using BCG/*M. tuberculosis*-specific T-cell populations or clones, or (ii) in vivo, either in animal models or in humans, using purified antigens or polymerized peptides as skin test reagents.

Subject to available reserves, samples of the IMMTUB mycobacterium-specific MAbs and the recombinant DNA library will be made available to qualified investigators by the contributing scientists, upon receipt of a short (one page) description summarizing the experiments to be conducted.

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