Indian J Med Res 88, July 1988, pp 13-17

Monoclonal antibodies against *Mycobacterium avium/intracellulare*

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Accepted March 16, 1988

Ten hybridoma cell lines prodacing monoclonaI antibodies (Mabs) against *M. avium/intracellulare* (Mai) serotype 8 were raised by the fusion of BALB/c mouse myeloma cells (SPZ) to spleen cells from immunized BALB/c mice. The specificity of the monoclonal antibodies was defined using their differing abilities to bind to sonicates from a range of mycobacterial species and strains. Tbe Mabs showed strain and species specificity. Three Mabs bound only to Mai serotype 8 and 1 Mab bound only to Mai serotypes 8 and 16, the only serotypes tested. The results indicate that Mabs specific for Mai species and serotypes can be produced. These could be useful for serodiagnostic and for epidemiological purposes.

Organisms of the *Mycobacterium avium/intracellulare* complex (Mai) are of fairly frequent occurrence in man, especially in countries such as India[1], where either they colonise the tracheal mucosa or more rarely are responsible for disease. Monoclonal antibodies (Mabs) specific for Mai could therefore be of value in the study of mycobacterial disease. Firstly, their high specificity might be used to subdivide species for epidemiological purposes. Secondly, the Mabs could form the basis of serodiagnostic tests specific for Mai infection, which is posing an increasing problem in immunocompromised persons, particularly those with AIDS. Thirdly, the Mabs might be used to isolate the specific epitopes from Mai antigen. These molecules could then be invaluable in testing the hypothesis that prior exposure to environmental Mai impairs the efficacy of BCG vaccination[2,3]. Mai is likely to be more important as an environmental immunogen than other mycobacteria, because it was the most frequent non-tuberculosis mycobacterium isolated from sputum in the south Indian region where BCG vaccination was found to give poor protection[1,4].

Material & Methods

Bacteria : M. avium/intracellulare serotype 8 (NCTC 10610) and serotype 16 (NCTC 10425) were used *M. tuberculosis* strains were the laboratory strain H37Rv (phage

type B), strain S1 (isoniazid-sensitive, phage type A) obtained before chemotherapy from a British patient and strain 7219 (isoniazid-sensitive; low virulence in the guineapig, with a rootindex of virulence of 0.57; phage type I) from a south Indian patient. Strains of *M. bovis* were Vallee (NCTC 5693) and BCG-Glaxo (both phage type A). Other bacteria were *M. scrofulaceum* (NCTC 10803), *M. kansasii* (NCTC 10268), *M. gordonae* (NCTC 10267), *M. flavescens* (NCTC 10271), *M. terrae* (NCTC 10856), *M. fortuitum* (NCTC 10394), *M. chelonei* (abscessus) (NCTC 10882) and *Escherichia coli* strain K12.

Antigen preparation : Antigens used for immunisation and screening were ultrasonic lysates of mycobacteria. The mycobacterial cultures were grown on the surface of liquid Sauton's medium until confluent colonies were just apparent. The cultures were then harvested and sonicated in phosphate buffered saline (Dulbecco 'A') in an ice bath for 15 min (in 5 sec bursts) using a Rinco Model MP ultrasonicator (Rinco Ultrasonics UK Ltd., London) at 70 per cent maximum intensity. After ultracentrifugation of the lysates at 100,000 g for 1 h (Kontron Instruments; Switzerland) the supernatants were sterilised by filtration (0.22 pm Millipore, SLGV membrane) and adjusted to a standard protein content as determined by a modified Lowry's method with tartrate replaced by citrate[5].

Immunisation and fusion : BALB/c mice were immunised ip with Mai serotype 8 antigen containing 100 µg protein with incomplete Freund's adjuvant on days 0 and 15. Ten days after the second inoculation, sera from the mice were tested for antibody activity against Mai serotype 8 using solid phase radioimmunoassay (RIA) as described previously[6]. Mice which showed high levels of antibody were selected and a third injection, without adjuvant, was given ip three days before harvesting the spleen cells for fusion.

Polyethylene glycol mediated fusions were done with the mouse myeloma cell line SP2[7]. The culture supernatants from numerous hybrids were initially tested by RIA against sonicates of Mai serotype 8 and *Esch. coli,* to determine the broad specificity of the clones. After two limiting dilutions, 14 clones were selected and fuller specificities were determined by testing their culture supernatants against sonicates of the 14 mycobacterial species and *Esch. coli.* Of these clones, 10 which showed diverse specificities were injected ip into pristane-primed mice to induce antibody-rich ascites and each ascitic fluid sample was also tested for specificity.

Characterisation studies : The monoclonal antibodies (Mabs) were further characterised by determining their immunoglobulin isotypes by immunofluorescent staining of the clones. Three clone supernatants were selected for immunoblotting to determine the approximate molecular weight of the antigenic proteins to which the Mabs bound and to confirm the specificities shown by solid phase RIA[8]. Blotting was against the mycobacterial sonicates that had been run on sodium dodecylsulphate-polyacrylamide slab gel electrophoresis (SDS-PAGE) under reducing conditions[9].

Results

The binding of ascitic fluid rich in Mab AI/1 to the antigen sonicate of Mai serotype 8 as well as the control binding to the plastic well is show in the Fig., and is representative of all of the curves obtained with the ascitic fluids, Based on such titrations, each ascitic

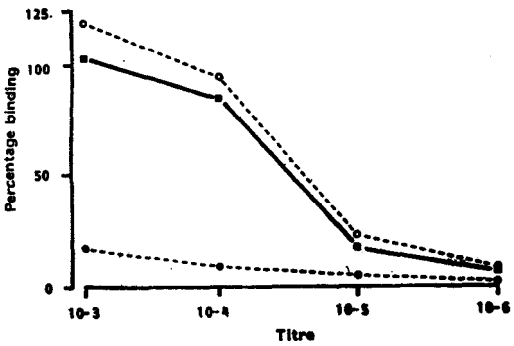

Fig. Titration curves of binding of monoclonal antibody AI/10 (ascitic fluid) to (a) sonicate of Mai serotype 8 (O–O), (b) to empty plastic wells (● – ●) and net binding (■ – ■ , a–b). Binding is expressed as a percentage in relation to a control well coated with normal mouse serum (100% reference).

fluid was diluted to an extent (to 10^{-4}; in Fig.) consistent with retaining net binding of antibody to Mai serotype 8 antigen that was at least 20 per cent of the values for normal mouse serum-coated wells *(i.e.,* percentage binding >20%). The ascitic fluid was then assayed at this dilution for cross-reactivity with other mycobacterial sonicates.

The binding patterns and antibody isotypes for ascitic fluids from 10 hybridomas are shown in the Table. The binding of these Mabs suggested 6 distinct patterns. The first is shown by a group, containing AI/4, AI/6 and AI/7, binding only to Mai serotype 8 and not to any of the other mycobacterial antigens including Mai serotype 16; AI/2 shows species specificity in that it bound only to the Mai strains tested and not even to the antigenically closely related *M. scrofulaceum;* the third pattern was shown by AI/5 and AI/8, which reacted only with Mai serotype 8 and, to a low degree, *M. tuberculosis* H37Rv; in contrast, AI/l bound slightly to *M. bovis* BCG in addition to Mai serotype 8 ; the fifth pattern is shown by AI/3, which bound to Mai serotype 8, *M. bovis* Vallee and *M. fortuitum.* AI/9 bound to about half to the mycobacterial sonicates tested and AI/10 reacted positively with all of them.

The binding patterns for all 10 Mabs were also assessed using undiluted supernatant from tissue culture wells and there was good agreement with results from ascitic fluids. Further, assays of subclones of AI/9 (to confirm purity of AI/9) were in close agreement with the results shown.

In immunoblotting, AI/10, which bound to all of the mycobacterial antigens tested by RIA, bound uniformly to a common antigen of approximately 45 kdaltons. AI/8, chosen for its RIA specificity for Mai serotype 8 and to *M. tuberculosis* H37Rv, bound selectively to an antigen of molecular weight of approximately 110 kdaltons that appeared to be present only in blots prepared from extracts of these two organisms. AI/7 bound only to an antigen of approximately 90 kdaltons in Mai serotype 8 blots.

Discussion

Discrimination between mycobacterial strains and species was achieved with 9 of the 10 Mabs (Table), though more strains should be tested before firm conclusions on specificity of these Mabs can be reached. Mabs that distinguish between Mai serotypes 4, 8 and 9 have recently been reported[10]. Of particular interest was the ability of most of our Mabs to differentiate between Mai serotype 8 and serotype 16. Our evidence is insufficient to conclude whether or not AI/4 and AI/6 bind to the Schaefer antigen of serotype 8 which is a small peptidoglycolipid[11]. In contrast, it seems unlikely that AI/7 reacts with the Schaefer antigen

Table. Specificity of monoclonal antibodies†

Antigen	Monoclonal antibody									
	AI/4	AI/6	AI/7	AI/2	AI/5	AI/8	AI/1	AI/3	AI/9	AI/10
M. avium/intracellulare (serotype 8)	41	78	31	31	21	16	24	41	54	100
M. intracellulare (serotype 16)	*	*	**	12	*	*	*	*	**	25
M. scrofulaceum	*	*	*	*	*	*	*	*	14	32
M. tuberculosis										
H37Rv	*	*	*	*	10	10	*	**	**	26
SI	*	*	**	*	*	*	*	*	**	20
7219	*	*	**	**	*	*	*	*	19	2.5
M. bovis										
Vallee	*	*	*	*	*	*	*	13	41	27
BCG	*	*	*	**	*	*	11	*	19	24
M. kansasii	*	*	*	*	*	**	*	*	**	38
M. flavescens	*	*	*	**	*	*	*	*	52	35
M. gordonae	*	*	*	*	*	*	*	*	**	39
M. terrae	*	*	*	*	**	*	*	**	*	30
M. fortuitum	**	*	*	*	*	**	*	16	52	30
M. chelonei	*	*	*	*	*	*	*	*	76	61
Esch. coli	*	*	*	*	*	*	*	*	*	*
Isotype	2a	2a	2b	2a	2b	3	2a	2a	1	1

† Percentage binding, defined as described in the text, is‘ shown only for positive reactions (binding of ≥ 10 %).
** 5–9.9 % binding; * < 5% binding

because immunoblotting revealed that this Mab binds to a 90,000 dalton molecule. Further, this antigen was detected as a single band with protein stains such as Coomassie blue and Amido black. It is nevertheless possible that it contains the antigenic carbohydrate residues present in the Schaefer antigen and that the Mab binds to this carbohydrate epitope.

Isolation of antigens with Mabs such as these would open two important avenues of research into the reasons for the relative failure of the BCG trial in south India. For example, a Mab such as AI/2 could be used to purify an antigen with an epitope shared between several serotypes of *M. avium/intracellulare* but absent in other species of mycobacteria. If this antigen was more specific for Mai than PPD B it could be useful as a skin test reagent or as a stimulant of circulating T lymphocytes to establish the incidence of exposure of individuals in south India to Mai. However, if this antigen was a large protein molecule it would probably bear many different epitopes, some of which could be present in other mycobacteria and thus compromise

the specificity of the AI/2 epitope. In this situation fractionation of the molecule might yield the specific epitope. Secondly, antigens isolated by Mabs AI/9 or AI/10 would bear epitopes which were shared between Mai, *M. bovis* BCG and *M. tuberculosis.* One of the hypotheses to explain the low protective value of BCG vaccination in south India, is that exposure to environmental mycobacteria, likely to be Mai, provides a degree of immunity which cannot be increased by subsequent BCG vaccination. An exploration of any interaction between shared epitopes and the specific epitope in protective cellular immunity might help to clarify the basis of success and failure in BCG vaccination.

Acknowledgment

Authors are grateful to Dr P.A. Jenkins, for advice and provision of *M. avium/intracellulare* strains. The first author was supported by a British Council TCTP fellowship.

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