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THE USE OF MONOCLONAL ANTIBODIES FOR THE CHARACTERIZATION AND PRODUCTION OF *MYCOBACTERIUM LEPRAE* ANTIGENS

J. IVANYI

MRC Tuberculosis and Related Infections Unit, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 OHS, England

Similar immunizations of mice and hybridoma technology were used by several investigators to raise monoclonal antibodies which identified a limited range of epitopes and antigenic molecules. Further studies would have the scope for revealing yet more novel structures. The existing MABs are agreed standard reagents, available to investigators and valuable for several applications. At least six epitopes specific for M. leprae *were defined in molecular terms. Monoclonal antibody based immunoassays proved to be invaluable for the screening of recombinant DNA clones and for the topographic study of individual epitopes. Purification of antigens using affinity chromatography requires further development of techniques whilst serology of leprosy is open for clinical and epidemiological evaluation.*

Immune responses to antigens of *M. leprae* play a key role in the pathogenesis of leprosy. Following infection, effective immunity can lead to self-healing; alternatively, the pronounced but ineffective lymphocyte and macrophage reactions can be manifested as tuberculoid leprosy, or the specific anergy of the lymphoid system may allow unlimited multiplication and spread of bacilli associated with lepromatous leprosy. It has been suspected, but not proved, that the spectrum of clinical and immunological reactions could be attributed to differences in the activated host lymphocyte repertoires in respect of the various antigenic constituents of the infecting organism (Ivanyi, 1986). Consequently the fundamental research aimed towards the definition of the antigenic structure of *M. leprae* is of great importance. Progress along this way was made by monoclonal antibodies (MAB) which had been raised during recent years by investigators in several laboratories. These reagents were evaluated in a WHO sponsored workshop, etablishing their corresponding specificities and basic properties (Engers, 1985). The purpose of this paper is to review the current position in the application of monoclonal antibodies to the various objectives of leprosy research.

Protein antigens – The antigenic molecules of *M. leprae* defined by monoclonal antibodies are listed in Table. These were determined by immunoblot analysis of the *M. leprae* soluble extract (MLSE) using SDS-polyacrylamide gel electrophoresis. When comparing the various MABs, competition for antigen binding between a pair of antibodies enabled to distinguish between distinct or overlaping specificities. Soluble extracts from several species of mycobacteria were tested for the definition of the binding specificity of the MABs. Species specific determinants (epitopes) were found on six proteins with molecular weights corresponding to 65kD, 36kD, 35kD, 28kD, 18kD and 12kD. In the case of the 35kD antigen, the epitope binding to MAB fails to renature from SDS and therefore MAB-based affinity chromatography was used for purification and identification of the molecular weight (Ivanyi et al., 1985). This molecule seems to differ from the 36kD protein as the former is contained in line 2, whereas the latter in line 7 defined by counterimmunoelectrophoresis (Harboe & Ivanyi, 1987). These two proteins probably differ also by being cytoplasmic or cell-wall origin, respectively. Epitopes cross-reactive with other species of mycobacteria have not yet been demonstrated on the 35kD, 18kD and 12kD molecules but their existence seems likely from studies based on polyclonal antisera. However, several cross-reactive epitopes were identified on the 65kD protein and at least one was detected on the 28kD antigen. The epitope of the 70kD protein shows a limited extent of cross-reactivity.

Glycolipid antigens – Chemical analysis and monoclonal antibodies (Young et al., 1984) identified an *M. leprae*-specific terminal disaccharide on the phenolic glycolipid (PGL-I) which represents a major constituent of the organism. Anti-PGL-I MABs differ in fine specificity as their binding can be abrogated by the removal of either a single (3,6-di-O-methyl glucose) terminal sugar or two sugar residues, while deletion of long chain fatty acids from the phthiocerol diester part of the molecule did

TABLE

Antigens of *M. leprae* defined by monoclonal antibodies

Molecular weight	Structure	Antibody code (isotype)	Binding to other mycobacteria	Reference****	Note
70kd	protein	L7(IgG)	limited	7	
65kd	protein	IIIE9(G1),IVD8(G1)	none	1	CIE***line 7
		IIH9(G2a)	broad	1	
		IIC8(G2b)	broad	1	
		ML30(G1)	broad	2	
30-40kd	protease-resistant*	ML34(IgM)	limited	2	Cell wall
		ML02(G3),ML09(G3)	limited	2	
		L1(IgM),L9(G3)	broad	7	
36kd	protein	F47-9(G1)	none	4	CIE line 7
35kd	protein	ML04(G1),ML03(G1),ML38(G1)	marginal**	2	CIE line 2 cytoplasm
28kd	protein	SA1.A1B(G1)	none	6	
		D2DG1)	broad	6	
18kd	protein	L5(G1),L6(IgM)	none	7	
12kd	protein	ML06(G1),ML10(G2a)	none	2	CIE line 11 cytoplasm
4.5-6kd	protease-resistant	L3(IgM)24(G2a)B6(G3)	broad	7	
15kd	phenolic glycolipid I	B8F(IgM)	none	3	surface capsule
		A1E	none	3	
		B9D	broad	3	
Arabinomannan			broad	5	cell wall

*Corresponding to lipoarabinomannan B (Brennan et al., 1986; D. Young, personal communication).
**RIA; limited cross-reactivity by nitrocellulose dot-blot (Engers, 1985).
***Crossed immunoelectrophoresis (Harbore & Ivanyi 1986; Engers et al. 1985).
****1. Gillis and Buchnan, 1982; 2. Ivanyi et al., 1983; 3. Young et al., 1984; 4. Klatser et al., 1984; 5. Miller & Buchanan, 1984; 6. Young et al., 1985; 7. Britton et al., 1986; 8. Engers et al., 1985.

not affect antibody binding. This antigen forms a surface capsule and has been found in large amounts in tissues and body fluids of multibacillary leprosy patients and infected armadillos. Detection of PGL-I in the sera of patients with lepromatous leprosy was achieved by a recently developed immunoassay (Young et al., 1985a, b, c). Test sera are applied as spots onto the polysulphone membrane and developed by binding with anti-PGL-I MAB followed by labelled anti-mouse immunoglobulin antibody. This test working with a sensitivity of 0.05 μg PGL-I per ml is applicable for the diagnosis of patients with multibacillary leprosy. Their identification is of the greatest epidemiological interest as they represent the source of infection to the susceptible contacts within communities at a risk. Detection of PGL-I may also be of interest for monitoring the effectiveness of chemotherapy as a convenient marker of its sterilising effect during management.

Another antigen of interest is the lipoarabinomannan B (LAM-B) (Hunter et al., 1986). This structure carries at least two distinct epitopes identified by MABs (Ivanyi et al., 1983; Britton et al., 1986). These epitopes cross-react with several other species of mycobacteria. It is of interest that anti-LAM-B antibody binds to intact *M. tuberculosis* bacilli, but not to *M. leprae* where it might be covered by PGL-I or by host-derived molecules; however binding is revealed following ultrasonication of the *M. leprae* bacilli (Praputpittaya & Ivanyi, 1985).

Recombinant DNA clones - *M. leprae* is not cultivable *in vitro* and the production of bacilli from infected armadillo tissues is restrictive. Hence, expression of individual antigens in recombinant DNA clones has been a major breakthrough in leprosy research (Young et al., 1985a, b, c). This was achieved by the insertion of mechanically sheared 1-71b DNA fragments with random endpoints into lambda gt11 phage vectors. Pooled and later individual MABs were used to probe the antigen expression of this DNA library in *E. coli* clones. Phage plaques were blotted onto nitrocellulose and developed

by immunoassay using several existing MABs. This approach demonstrated the exquisite advantage of MABs over the use of cross-absorbed polyclonal antisera for the identification of recombinant clones expressing species-specific antigens. The *M. leprae* DNA library contains 2.5 x 10^6 individual recombinant phage comprehensively representing the DNA of the bacillus and was amplified to a titre 2 x 10^{11} plaques. All clones isolated with monoclonal antibodies directed against distinct proteins apparently align with single linear DNA segments. However, conformational epitopes would not be detected by this method.

Competitive binding assays showed that monoclonal antibodies recognise at least 14 topographically distinct epitopes on the 65kD molecule (Buchanan et al., 1987). A DNA sublibrary was prepared from fragments of the 65kD gene (Mehra et al., 1986). These clones were screened by MABs to 6 epitopes and their amino acid sequences were determined on the basis of minimum overlap among adjacent clones which bound with the same antibody.

Affinity chromatography – Despite the monospecificity of MABs, their application for the purification of antigens from soluble extracts of *M. leprae* or from recombinant *E. coli* lysates has met with only partial success (Ivanyi et al., 1985). This is attributable probably to complexing between the constituents within these extracts. Alternatively, several antigens may remain associated with cell-wall derived fragments. The general experience has been that MAB-based affinity columns perform at a low capacity, produce low yields and it is difficult to obtain satisfactory purity of antigens without contaminating constituents.

Columns of CNBr-activated Sepharose 4B, coupled with 60mg of anti-12kD (ML06) MAB were used for the purification of the 12kD antigen from MLSE. Soluble extracts were treated with protease inhibitors and spun 30 min. at 100,000 g prior to separation. Elution of antigen with 3M NaSCN yielded about 50% of antigen contained in the original soluble extract. Similar results were obtained using the anti-35kD (ML04) MAB coupled to protein A-Sepharose 4B and 2.5M NaI as the eluting buffer (Ivanyi et al., 1985). Each MAB and corresponding antigen, require individual evaluation in the selection of the most suitable conditions for coupling and elution from the column. Additional development of techniques is required for scaling up the fractionation procedures.

Serological studies – It has been demonstrated in several previous studies that patients with lepromatous leprosy produce high antibody levels to *M. leprae* (Melsom, 1983). Hence, serological diagnosis is of interest as an adjunct to the current means of case finding and particularly suitable for large-scale screening of populations in leprosy endemic areas. Development of a useful test depends on the identification of epitopes which are specific for *M. leprae* as well as strongly immunogenic in leprosy patients. Testing for antibodies to individual epitopes without antigen purification was achieved by the competition test, whereby the binding of radio – or enzyme – labelled MABs to MLSE-coated microtitre plates is inhibited only by sera containing the same specificities as the labelled MAB probe (Hewitt et al., 1982). By this type of analysis, it was found that sera from essentially all LL patients have high levels of anti-35kD but do not contain any anti-12kD antibodies (Sinha et al., 1983). Subsequent studies using the competition assay reported that antibody levels to several other antigens were elevated to various degrees in both lepromatous and tuberculoid leprosy (Klatser et al., 1985; Britton et al., 1986). It has also been reported that sera from a small number of healthy leprosy contacts contain low levels of anti 35kD antibodies (Sinha et al., 1985; Ashworth et al., 1986). Although this approach is of interest for the possible prognostic value of serology, the results cannot be evaluated without more extensive long-term follow up studies. Anti 35kD antibody formation was found also in volunteers in Norway who were given the killed *M. leprae* vaccine (Gill et al., 1986). This antibody response was induced by 1.5 – 5 x 10^8 but not by lower numbers of bacilli and developed consistently only 6 months after vaccination.

Serological studies were performed also using the *M. leprae* specific PGL-I and its terminal disaccharide as solid-phase coating antigen in an ELISA test (Cho et al., 1983; Brett et al., 1983). This technique has the advantage in the availability of the synthetic disaccharide hapten and in the greater popularity of binding over the competition assays. However, non-specific adsorbtion of serum IgM, particularly when in immune complex, may interfere with the readings of weak positive values in the PGL-I binding assay.

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