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Screening of a Recombinant Mycobacterial DNA Library with Polyclonal Antiserum and Molecular Weight Analysis of Expressed Antigens

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A λgt11 expression library containing recombinant DNA from *Mycobacterium tuberculosis* was screened using hyperimmune anti-*M. tuberculosis* rabbit serum. The majority (22 of 29) of the recombinant clones selected by using polyclonal serum expressed three antigens that were previously identified by using mouse monoclonal antibodies, thus indicating the immunodominance of these proteins. Western blot analysis of the recombinant clones demonstrated that expression of these antigens is frequently independent of the formation of beta-galactosidase fusion proteins. The molecular weight of each expressed antigen can vary between clones and is not necessarily identical to that found in mycobacterial extracts.

The cloning and expression of DNA from *Mycobacterium tuberculosis* and *Mycobacterium leprae* using the λgt11 system represents a major technical advance in the study of mycobacterial antigens (18, 19). This technique not only allows the potential production of individual proteins in large quantities but also provides a means of identifying immunologically important determinants within these molecules (12). Mycobacterial antigens expressed by recombinant clones have begun to be used for analysis of the human T-cell repertoire (5, 11, 13), and this is clearly an important approach to the development of novel skin test reagents and potential vaccines to combat mycobacterial infection.

The mycobacterial antigens expressed in the λgt11 system have been detected using monoclonal antibodies (18, 19; H. D. Engers and Workshop Participants, Letter, Infect. Immun. **51**:718–720, 1986). While polyclonal antiserum was used to screen an alternative library of *Mycobacterium bovis* BCG DNA (14), the clones analyzed in detail also expressed an antigen already defined by monoclonal antibodies (14; Engers et al., Letter, 1986). Crossed-immunoelectrophoresis analysis has demonstrated a large number of antigens in mycobacterial extracts (3), and it is surprising that only a few proteins are recognized by monoclonal antibodies (Engers et al., Letter, 1986; H. D. Engers and Workshop Participants, Letter, Infect. Immun. **48**:603–605, 1985). The production of monoclonal antibodies of overlapping specificities in different laboratories has led to the suggestion that these proteins are the major antigens of mycobacteria (19; Engers et al., Letter, 1985; Engers et al., Letter, 1986), but it can alternatively be argued that this finding represents an artifact of the procedures used for generation of the monoclonal antibodies and that these antibodies cover only the limited repertoire of the BALB/c mice used for hybridoma production. We have therefore used a polyclonal hyperimmune rabbit serum to screen the λgt11 *M. tuberculosis* recombinant DNA library and have compared the results with earlier screens using mouse monoclonal antibodies. In addition, we have analyzed the molecular weight of the expressed antigens by Western blotting and report expression of several antigens in unexpected molecular-weight forms.

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MATERIALS AND METHODS

Screening of recombinant library. Construction of the *M. tuberculosis* library in λgt11 has been described previously (18). Anti-*M. tuberculosis* hyperimmune serum, produced by repeated subcutaneous immunization of rabbits with *M. tuberculosis* H37Rv culture filtrate, was generously provided by J. Bennedsen (Statens Seruminstitut, Copenhagen, Denmark). The library was screened using the rabbit anti-*M. tuberculosis* serum absorbed with *Escherichia coli*, and recombinant phage producing positive signals were cloned by plaque purification (18). Clones Y3143, Y3272, and Y3151 were selected from the same library using appropriate monoclonal antibodies for screening as described previously (18; Engers et al., Letter, 1986).

Clones were subsequently screened by plaque blotting (18) using hyperimmune rabbit anti-*M. bovis* BCG antibody (Dako Ltd., High Wycombe, Buckinghamshire, U.K.) absorbed with *E. coli*, and the following monoclonal antibodies: TB78 (IT13 [4, 8; Engers et al., Letter, 1986]), SA2.D5H (IT31 [Engers et al., Letter, 1986]), 51A (IT11 [Engers et al., Letter, 1986]), L7 (1), TB71 (IT23 [4, 8; Engers et al., Letter, 1986]), TB72 (IT15 [4, 8; Engers et al., Letter, 1986]), SA1.D2D (IT17 [17; Engers et al., Letter, 1986]), TB23 (IT19 [4, 8; Engers et al., Letter, 1986]), and TB68 (IT20 [4, 8; Engers et al., Letter, 1986]). The secondary antibodies used for detection were peroxidase-conjugated affinity-purified goat anti-mouse and anti-rabbit immunoglobulins supplied by BioRad Laboratories (Richmond, Calif.). The substrate usedforcolordevelopmentwas3,3′-diaminobenzidinedihydrochloride (Sigma Chemical Co., St. Louis, Mo.).

Western blot analysis. Lysogenic strains were prepared from phage clones in *E. coli* Y1089 (7). Lysogens were inoculated into 10 ml of L broth (7) and grown in universal containers at 32°C with vigorous aeration to an A_{600} of 0.5. Cultures were then induced by incubation for 20 min in a water bath at 45°C, followed by addition of isopropyl-β-D-thiogalactopyranoside to 10 mM and further incubation at 38°C for 1 h with shaking. Bacteria were harvested by centrifugation and suspended in 1 ml of phosphate-buffered saline (pH 7.2). An equal volume of gel sample buffer (10) was added to a portion of the resuspended pellet, and the

TABLE 1. Analysis of antigens expressed by recombinant clones[a]

Clone no.	Recognition by monoclonal antibody:	Fusion protein (M_r, 10^3)	Expressed antigen (M_r, 10^3)
Y3107	TB78		65
Y3110	TB78	124	65
Y3113	TB78	132	65
Y3114	TB78	126	65
Y3115	TB78	123	68
Y3117	TB78	133	65
Y3119	TB78		65
Y3126	TB78	120	65
Y3118	TB78	NT	NT
Y3101	IT11		81
Y3103	IT11		81
Y3105	IT11		52
Y3111	IT11	141	71
Y3130	IT11		71
Y3102	IT11	NT	NT
Y3122	IT11	NT	NT
Y3120	TB68		25
Y3121	TB68		25
Y3124	TB68		25
Y3128	TB68		25
Y3123	TB68	NT	NT
Y3129	TB68	NT	NT
Y3104			
Y3106			
Y3108		119	
Y3112		170	170
Y3116			
Y3125			100
Y3127			

[a] Recognition by monoclonal antibodies was established by plaque blotting as described in the text. The molecular weight of fusion proteins was estimated as described in the legend to Fig. 1, and the molecular weight of expressed antigens was determined by Western blotting as described for Fig. 2. NT, Not tested; —, not detected.

mixture was heated for 2 min in a boiling-water bath. Samples (10 μl) were applied to sodium dodecyl sulfate (SDS)-polyacrylamide gels (12% [wt/vol] or 6% [wt/vol], final acrylamide concentration; 8) and subjected to electrophoresis at 15 mA per gel for 50 min using a mini-gel system (Hoeffer Scientific Instruments, San Francisco, Calif.). Blots were prepared on nitrocellulose membranes (BioRad Laboratories) by electroblotting gels at 50 V for 1 h (15). Blots were washed with 0.2% Triton X-100 in PBS, and nonspecific binding was blocked by carrying out subsequent incubations in the presence of 5% dried milk powder in PBS-Triton. Subsequent development of Western blots with monoclonal and polyclonal antibodies was performed as described previously (16). Molecular weights were estimated by running known molecular-weight standards (Sigma) on the gels. For visualization of total protein, blots were stained with amido black (15).

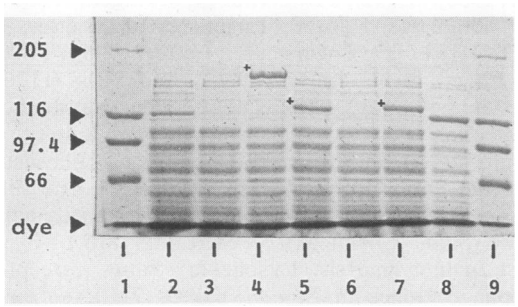

FIG. 1. Analysis of total protein profiles from recombinant clones. Lysogens prepared from recombinant clones were grown and induced as described in the text, and samples were analyzed by SDS-PAGE using 6% (wt/vol) final acrylamide concentration. Proteins were transferred to nitrocellulose by electroblotting and stained with amido black. Prominent fusion proteins (lanes 4, 5, and 7) are marked with a cross. Lanes: 1 and 9, molecular weight markers; 2 and 8, control lysogens with λgt11 containing no insert DNA; 3, clone Y3107; 4, clone Y3143; 5, clone Y3114; 6, clone Y3101; 7, clone Y3110.

RESULTS

Plaque blotting. A total of 5×10^5 phage from the *M. tuberculosis* recombinant DNA library were screened by plaque blotting (18) using a hyperimmune rabbit antiserum to *M. tuberculosis*. Twenty-nine positive phage were cloned by plaque purification and were subsequently found to be positive when screened with Dako anti-*M. bovis* BCG antibody. This is consistent with previous reports of widespread cross-reactivity between these two species of mycobacteria (3). Nine of the clones were positive when screened with monoclonal antibody TB78 directed to the 65-kilodalton (kDa) antigen of *M. tuberculosis* (Table 1). A further seven clones were positive with monoclonal antibody IT11 (71-kDa antigen), and six were positive with monoclonal antibody TB68 (14-kDa antigen). The remaining seven clones were negative with all of the monoclonal antibodies tested.

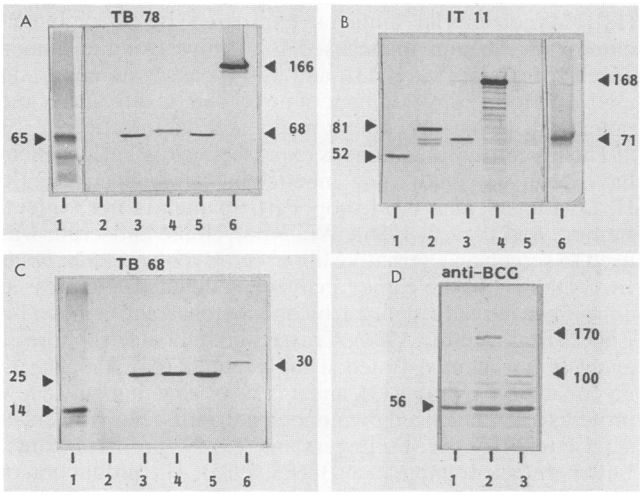

FIG. 2. Analysis of recombinant clones by Western blotting. Lysogens prepared from recombinant clones were grown and induced as described in the text, and samples were analyzed by SDS-PAGE using 6% (panels A, B, and D) or 12% (panel C) acrylamide gels. Proteins were transferred to nitrocellulose by electroblotting, and membranes were reacted with monoclonal antibodies TB78 (A), IT11 (B), or TB68 (C), or rabbit anti-*M. bovis* BCG (D). Molecular weights were estimated by comparison with standard protein markers. (A) TB78 (65-kDa antigen). Lanes: 1, *M. tuberculosis* extract; 2, control lysogen with λgt11 containing no insert DNA; 3, clone Y3107; 4, clone Y3115; 5, clone Y3110; 6, clone Y3143. (B) IT11 (71-kDa antigen). Lanes: 1, clone Y3105; 2, clone Y3101; 3, clone Y3111; 4, clone Y3272; 5, control lysogen with λgt11 containing no insert DNA; 6, *M. tuberculosis* extract. (C) TB68 (14-kDa antigen). Lanes: 1, *M. tuberculosis* extract; 2, control lysogen with λgt11 containing no insert DNA; 3, clone Y3120; 4, clone Y3121; 5, clone Y3128; 6, clone Y3151. (D) Polyclonal antiserum. Lanes: 1, control lysogen with λgt11 containing no insert DNA; 2, clone Y3112; 3, clone Y3125.

Protein profiles. Lysogens were prepared from 24 of the 29 clones, and the total protein profile from cell extracts was analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) on 6% gels. Prominent protein bands in the molecular weight range of 116,000 to 170,000, indicative of high-level production of beta-galactosidase fusion proteins, were seen for some but not all of the clones. The molecular weights of distinct fusion proteins are recorded in Table 1, and Fig. 1 shows examples of lysogens with and without distinct fusion proteins.

Western blotting. Blots prepared from lysogens were screened with anti-*M. bovis* BCG antibody and with appropriate monoclonal antibodies. Of the nine prominent fusion proteins expressed by the clones, only one (Y3112) was recognized by anti-*M. bovis* BCG antibody (Fig. 2D), and none was found to react with any of the monoclonal antibodies.

Eight of the clones recognized by monoclonal antibody TB78 were analyzed by Western blotting. In all but one, the antigen reacting with monoclonal and polyclonal antibodies had a molecular weight of approximately 65,000, identical to that of the major protein recognized by this antibody in *M. tuberculosis* extracts (Engers et al., Letter, 1986). Clone Y3115 expressed an antigen with a slightly higher molecular weight of 68,000. Figure 2A illustrates representative clones expressing the 65- and 68-kDa proteins as well as an example of a clone (Y3143) expressing the same determinant in the form of a fusion protein.

Three distinct molecular-weight forms of the protein recognized by monoclonal antibody IT11 were observed (Fig. 2B). Two clones expressed an antigen with molecular weight identical to that seen in *M. tuberculosis* extracts (71,000), while two expressed a higher-molecular-weight form (81,000) and one expressed a lower-molecular-weight product (52,000). Representatives of the three molecular weights are shown in Fig. 2B along with an example of the same determinant expressed as a fusion protein. Identical results were observed when the same set of clones was screened with monoclonal antibody L7, originally characterized as directed towards a 70-kDa antigen shared by *M. tuberculosis* and *M. leprae* (1).

All of the clones recognized by monoclonal antibody TB68 expressed a 25-kDa protein in contrast to the 14-kDa antigen recognized in *M. tuberculosis* extracts (Fig. 2C). Screening of other recombinant clones selected by TB68 antibody has shown expression of proteins at 25 kDa and also at 30 kDa (e.g., clone Y3151, shown in Fig. 2C), but as yet we have not found this determinant in the form of a fusion protein.

All of the antigens recognized by monoclonal antibodies were also clearly seen by development of blots with anti-*M. bovis* BCG antibody. Of the remaining seven clones, two expressed antigens which were weakly recognized by the polyclonal antiserum after Western blotting (Fig. 2D). Clone Y3112 expressed an antigenic fusion protein, and a protein of molecular weight 100,000 was detected in clone Y3125. In spite of extensive absorption of the anti-*M. bovis* BCG antibody, a prominent cross-reactivity with *E. coli* (particularly with a protein of molecular weight 56,000) was observed when this antiserum was used on Western blots at the high concentration required to detect these two recombinant proteins (Fig. 2D).

DISCUSSION

A striking result of this study is that, although a polyclonal antiserum was used for initial screening of the library, 22 of the 29 clones selected expressed three antigens that had been previously identified by using monoclonal antibodies. After immunization with crude mycobacterial extracts, monoclonal antibodies specific for each of these three antigens have been generated independently in separate laboratories (1, 4, 6, 9; Engers et al., Letter, 1985; Engers et al., Letter, 1986), and published reports indicate occurrence of monoclonal antibodies to the 65-kDa antigen of mycobacteria at a particularly high frequency (4, 6, 9; Engers et al., Letter, 1985; Engers et al., Letter, 1986). The results of the present study support the suggestion that the antigens defined by mouse monoclonal antibodies are immunodominant as regards the antibody response to mycobacterial proteins.

In a previous study using a pool of monoclonal antibodies for screening the *M. tuberculosis* library, the same three antigens accounted for 139 of 162 clones selected (Engers et al., Letter, 1986), suggesting that expression of these proteins in *E. coli* may occur at a particularly high frequency. The λgt11 expression system is designed to allow production of antigenic determinants as fusion proteins linked to beta-galactosidase (7), but analysis of the clones selected in this study demonstrated expression of nonfusion proteins often with molecular weights comparable to those of the same antigens in *M. tuberculosis*. In only one of the clones studied in detail was an antigenic fusion protein demonstrated, although clones expressing the same determinants as fusion proteins have been found during screening of the library with the appropriate monoclonal antibodies (e.g., Y3143 and Y3272). Expression in *E. coli* as a free protein has been reported for the 65-kDa antigen (14), and the hypothesis that the mycobacterial promoter for this protein can be recognized by *E. coli* transcription systems (14) could also be valid for the 71- and 14-kDa antigens. Recognition of a mycobacterial translation initiation signal on an mRNA molecule with transcription remaining under the control of the beta-galactosidase promoter would be an alternative explanation for the results reported here.

If the initiation signals for these particular proteins are indeed suitable for recognition by *E. coli* (in contrast to several other mycobacterial enzymes which have been studied [2]), then their expression in a recombinant DNA system would be independent of the orientation and reading frame of the insert. This effect could then contribute to the high frequency with which clones expressing these proteins are picked up. On the other hand, the method by which the λgt11 library was generated, using a large number of fragments with random endpoints, would be expected to make this effect less pronounced since the number of inserts with start points within the gene would be very high compared with those containing the initiation portions. The relative importance of these two factors in determining the frequency with which particular clones are detected will depend on the nature of the epitope recognized. For an epitope consisting of a short linear sequence of amino acids, it would be expected that fusion proteins would occur most frequently, but if a complex conformational determinant is recognized by the antibody used for screening, then the type of whole-gene transcript resulting from recognition of naturally occurring initiation sequences may provide a more satisfactory antigen. Although monoclonal antibodies to the 65-kDa protein have been shown to recognize linear determinants (12), the epitopes recognized by antibodies in the polyclonal serum have not been characterized.

In spite of being present as free proteins in *E. coli*, it was found that the molecular weight of expressed antigens was not an accurate guide to their identification since different

molecular-weight forms were found in different clones. Low-molecular-weight products could result from loss of the terminal region of a protein due to cloning of a partial gene in the insert; the 52-kDa protein (clone Y3105) recognized by anti-71-kDa monoclonal antibody may be an example of such an event. Several possible explanations can be considered for the observed expression of antigens in recombinant clones with molecular weights higher than those found in extracts from *M. tuberculosis*. If expression is under the control of a promoter signal within the insert DNA, then it is possible that readthrough to adjacent genes in the vector could result in formation of unusual "fusion" proteins with the mycobacterial antigen linked to a portion of the beta-galactosidase molecule. Fusion with the C-terminal fragment of beta-galactosidase produced by cloning in the *Eco*RI site, for example, could result in an increase of about 1,700 in molecular size, and this may explain the formation of a 68-kDa antigen in clone Y3115. Alternatively, higher-molecular-weight proteins would be found in *E. coli* if the antigens seen in mycobacterial extracts arise from posttranslational processing of original high-molecular-weight gene products. Monoclonal antibodies recognizing epitopes of the 65-kDa antigen generally bind to multiple molecular weight bands in mycobacterial extracts (9; Engers et al., Letter, 1985), but expression in *E. coli* demonstrates that the original gene product forms only a single band on SDS-PAGE. Posttranslational proteolysis of the 65-kDa protein in *M. tuberculosis* would be a consistent explanation for these results. Similarly, lower-molecular-weight forms of the 14-kDa antigen can be seen in culture filtrates of *M. tuberculosis* reacted with monoclonal antibody TB68, and it is possible that the 14-kDa protein itself is produced by partial breakdown of an original 25-kDa molecule such as that found in the recombinant clones. Immunization of mice with *E. coli* lysates containing the 25-kDa protein results in antisera giving a single strong band at 14,000 on Western blots with *M. tuberculosis* extracts (K. Sharp, D. Young, and J. Ivanyi, unpublished results), indicating that the remaining portion of the molecule either is immunologically silent or else is absent from the *M. tuberculosis* extract. Detailed restriction mapping and sequence analysis of open reading frames in the mycobacterial DNA inserts will be required to fully understand the origin of the different molecular-weight proteins expressed by these clones.

Seven of the clones selected with the polyclonal rabbit antiserum expressed proteins which were not recognized by any of the monoclonal antibodies tested. The inserts in these clones may therefore code for mycobacterial proteins distinct from the set recognized by the monoclonal antibodies tested (71, 65, 38, 19, and 14 kDa). Clone Y3125 expressed a 100-kDa product, although, as illustrated by the results discussed above, it cannot be assumed that this is the natural molecular weight of the antigen in *M. tuberculosis*. Clone Y3112 expressed an antigenic fusion protein, but again we have no information about the nature of this antigen in mycobacteria. The remaining five clones did not express antigens which could be detected by Western blotting. It is possible that the determinants recognized by the rabbit antiserum in these clones are dependent on antigen conformation and may have been destroyed by the denaturation step during SDS-PAGE. Further characterization using immunoprecipitation techniques and analysis of insert DNA will be important in defining the antigens expressed by each of these clones.

In conclusion, screening of an *M. tuberculosis* recombinant DNA library with monoclonal or polyclonal antibodies results in preferential selection of clones expressing one of three dominant mycobacterial antigens. These antigens are all capable of being expressed as free proteins rather than as fusion proteins and may therefore be under the control of promoters other than that of the beta-galactosidase gene. Each of the antigens can be expressed in different molecular-weight forms in different clones.

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LITERATURE CITED

1. **Britton, W. J., L. Hellqvist, A. Basten, and R. L. Raison.** 1985. *Mycobacterium leprae* antigens involved in human immune responses. I. Identification of four antigens by monoclonal antibodies. J. Immunol. **135**:4171–4177.
2. **Clark-Curtiss, J. E., W. R. Jacobs, M. A. Docherty, L. R. Ritchie, and R. Curtiss.** 1985. Molecular analysis of DNA and construction of genomic libraries of *Mycobacterium leprae*. J. Bacteriol. **161**:1093–1102.
3. **Closs, O., M. Harboe, N. H. Axelsen, K. Bunch-Christensen, and M. Magnusson.** 1980. The antigens of *Mycobacterium bovis* strain BCG studied by crossed-immunoelectrophoresis: a reference system. Scand. J. Immunol. **12**:249–263.
4. **Coates, A. R. M., B. W. Allen, J. Hewitt, J. Ivanyi, and D. A. Mitchison.** 1981. Antigenic diversity of *Mycobacterium tuberculosis* and *Mycobacterium bovis* detected by means of monoclonal antibodies. Lancet **ii**:167–169.
5. **Emmrich, F., J. Thole, J. van Embden, and S. H. E. Kaufmann.** 1986. A recombinant 65 kilodalton protein of *Mycobacterium bovis* Bacillus Calmette-Guerin specifically stimulates human T4 clones reactive to mycobacterial antigens. J. Exp. Med. **163**: 1024–1029.
6. **Gillis, T. P., and T. M. Buchanan.** 1982. Production and partial characterization of monoclona' antibodies to *Mycobacterium leprae*. Infect. Immun. **37**:172–178.
7. **Huynh, T. V., R. A. Young, and R. W. Davis.** 1985. Construction and screening cDNA libraries in lambda gt10 and gt11, p. 49–78. *In* D. M. Glover (ed.), DNA cloning, vol. I. IRL Press, Oxford, U.K.
8. **Ivanyi, J., J. A. Morris, and M. Keen.** 1985. Studies with monoclonal antibodies to mycobacteria, p. 59–90. *In* A. J. L. Macario and E. C. Macario (ed.), Monoclonal antibodies against bacteria, vol. I. Academic Press, Inc., New York.
9. **Ivanyi, J., S. Sinha, R. Aston, D. Cussel, M. Keen, and U. Sengupta.** 1983. Definition of species-specific and cross-reactive antigenic determinants of *Mycobacterium leprae* using monoclonal antibodies. Clin. Exp. Immunol. **52**:528–536.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Lamb, J. R., J. Ivanyi, A. Rees, R. A. Young, and D. B. Young.** 1986. The identification of T cell epitopes in *Mycobacterium tuberculosis* using human T lymphocyte clones. Lepr. Rev. **57**(Suppl. 2):131–137.
12. **Mehra, V., D. Sweetser, and R. A. Young.** 1986. Efficient mapping of protein antigenic determinants. Proc. Natl. Acad. Sci. USA **83**:7013–7017.
13. **Mustafa, A. S., H. K. Gill, A. Nerland, W. J. Britton, V. Mehra, B. R. Bloom, R. A. Young, and T. Godal.** 1986. Human T-cell clones recognise a major *M. leprae* protein antigen expressed in *E. coli*. Nature (London) **319**:63–66.
14. **Thole, J. E. R., H. G. Dauwerse, P. K. Das, D. G. Groothius, L. M. Schouls, and J. D. A. van Embden.** 1985. Cloning of *Mycobacterium bovis* BCG DNA and expression of antigens in *Escherichia coli*. Infect. Immun. **50**:800–806.
15. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci.

USA **76**:4350–4354.

16. **Young, D., L. Kent, A. Rees, J. Lamb, and J. Ivanyi.** 1986. Immunological activity of a 38-kilodalton protein purified from *Mycobacterium tuberculosis*. Infect. Immun. **54**:177–183.
17. **Young, D. B., M. J. Fohn, S. R. Khanolkar, and T. M. Buchanan.** 1985. Monoclonal antibodies to a 28,000 mol. wt. protein antigen of *Mycobacterium leprae*. Clin. Exp. Immunol. **60**:546–552.
18. **Young, R. A., B. R. Bloom, C. M. Grosskinsky, J. Ivanyi, D. Thomas, and R. W. Davis.** 1985. Dissection of *Mycobacterium tuberculosis* antigens using recombinant DNA. Proc. Natl. Acad. Sci. USA **82**:2583–2587.
19. **Young, R. A., V. Mehra, D. Sweetser, T. Buchanan, J. Clark-Curtiss, R. W. Davis, and B. R. Bloom.** 1985. Genes for the major protein antigens of the leprosy parasite. Nature (London) **316**:450–452.