JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1986, p. 446–451
0095-1137/86/030446-06$02.00/0

Vol. 23, No. 3

Interspecies Reactivity of Five Monoclonal Antibodies to *Mycobacterium tuberculosis* as Examined by Immunoblotting and Enzyme-Linked Immunosorbent Assay

ÅSE B. ANDERSEN,[1] ZENG-LIN YUAN,[2] KAARE HASLØV,[3] BODIL VERGMANN,[1] AND JØRGEN BENNEDSEN[1]*

Tuberculosis[1] and Tuberculin[3] Departments, Statens Seruminstitut, DK-2300 Copenhagen S, Denmark, and National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Temple of Heaven, Peking, People's Republic of China[2]

Received 23 July 1985/Accepted 5 December 1985

Five different murine monoclonal antibodies (MAbs) to *Mycobacterium tuberculosis* were examined for degree of cross-reactivity with other mycobacterial species by enzyme-linked immunosorbent assay and immunoblotting. One MAb reacted solely with *M. tuberculosis* and *M. bovis* BCG. Two of the MAbs reacted with all mycobacterial species examined, whereas two MAbs demonstrated a limited reactivity pattern. The epitopes are located on molecules susceptible to protease treatment, and two of these molecules possess concanavalin A-binding moieties. Two of the antigens defined by these five MAbs are present in tuberculin purified protein derivative.

Numerous approaches have been made to fractionate antigens of *Mycobacterium tuberculosis* to isolate and define biologically active components (3). So far, it has not been possible by classical physical-chemical methods to isolate species-specific antigens. Hybridoma technology has made it possible to identify single antigenic determinants, which permitted a powerful new approach to the characterization of antigens. In the past 5 years, several monoclonal antibodies (MAbs) have been reported (1, 2, 4, 6, 9, 10).

MAbs to mycobacterial antigens may prove useful in several lines of work. Knowledge of the immunological role of single antigens is a prerequisite to the inclusion of the antigen(s) in potential synthetic vaccines. Using MAbs for immunosorbent chromatography has made it possible to purify quantitative amounts of antigen. In one report, antigen so purified was tested for T-cell-stimulating activity (8). Detection of mycobacterial antigen in clinical specimens is another object of interest. Selected MAbs may be valuable tools in taxonomic analyses.

A sensitive, reliable interspecies analysis is an important part of the characterization of a MAb and is necessary for the evaluation of its possible usefulness. In this report, the degree of cross-reactivity to other mycobacteria of five different MAbs to *M. tuberculosis* were examined in a direct enzyme-linked immunosorbent assay (ELISA) and by immunoblotting analysis.

MATERIALS AND METHODS

Antigen production. The mycobacterial species used in this study were part of a series submitted by the International Working Group on Mycobacterial Taxonomy 1966. The series included *M. tuberculosis* H37Rv, *M. bovis* BCG, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. marinum*, and *M. fortuitum*. The bacteria were kept lyophilized until this work was undertaken. *M. tuberculosis* I95 was isolated from a Danish patient and selected as an intermediate type with respect to morphology and biochemistry. The bacteria were grown on Sauton medium and separated from the culture medium on a Buchner funnel. The bacteria were kept at −20°C until further processing.

CCF. Concentrated culture filtrate (CCF) was prepared as follows. Antigenic material in the culture medium was recovered by ammonium sulfate precipitation (80%). The precipitates were collected by centrifugation and dissolved in phosphate-buffered saline (PBS) (pH 7.4). Reprecipitation was done twice, and the final precipitate was dissolved and dialyzed in PBS. Insoluble material was removed by centrifugation at 40,000 × *g* for 2 h. The supernatant was filtered through 0.2-μm (pore size) membranes, and the protein concentration was adjusted to 5 mg/ml as measured by A_{280}. The protease inhibitor phenylmethylsulfonyl fluoride (Boehringer GmbH, Mannheim, Federal Republic of Germany) was added to a final concentration of 10 mM.

BPE. Bacterial press extract (BPE) was prepared as follows. The bacteria were ruptured in a French pressure cell press at 14,000 lb/in.^{2} and suspended in PBS (8 ml/gram of bacteria) containing 0.5% Tween 20. Iodoacetamide (0.12 M) and Trasylol (1%) (Boehringer) was added to prevent proteolytic degradation. Insoluble material was removed by centrifugation at 40,000 × *g* for 1 h. Protein concentration was measured by a method described by Markwell et al. (7).

CCWE. Insoluble material from BPE preparations, believed to be rich in cell walls and membranes, was rinsed in PBS and suspended in 20 mM Tris buffer (pH 6.8) containing 2% sodium dodecyl sulfate (SDS) and 5% β-mercaptoethanol (3 ml of buffer per g of bacteria), boiled for 20 min, and subjected to sonication. Insoluble remains were removed by centrifugation, and the supernatant was referred to as crude cell wall extract (CCWE).

Nocardia asteroides was grown on a Sabouraud dextrose agar slant. *Escherichia coli* C600 was grown in tryptone broth. Freeze-dried *Rhodococcus erythropolis* N41, originally grown on Long synthetic medium, was a generous gift from S. D. Chaparas, U.S. Food and Drug Administration, Bethesda, Md. The bacteria were suspended and sonicated in 20 mM Tris buffer (pH 6.8). SDS and β-mercaptoethanol were added to final concentrations of 2 and 5%, respectively,

* Corresponding author.

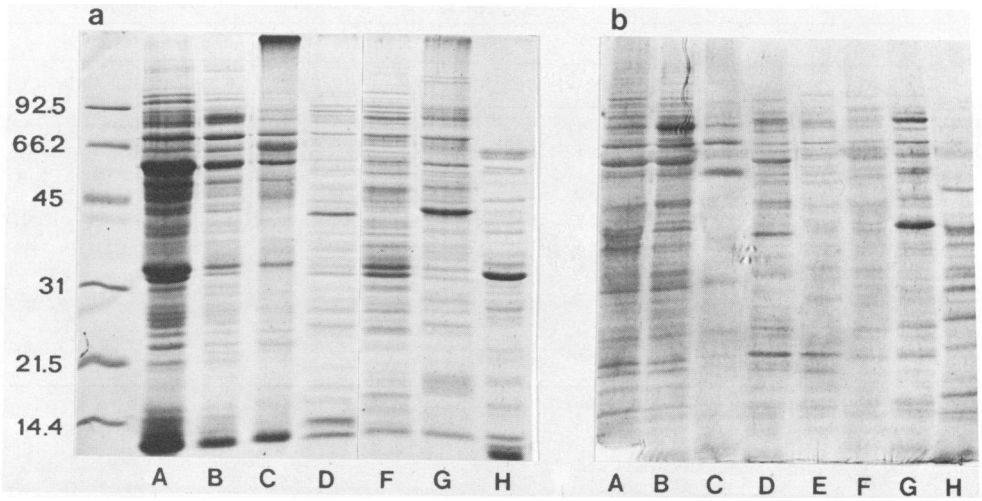

FIG. 1. CCFs (panel a) and BPEs (panel b) from different mycobacterial strains separated in a 12.5% polyacrylamide gel and stained with Coomassie brilliant blue. Lanes: A, *M. tuberculosis* H37Rv; B, *M. tuberculosis* I95; C, *M. bovis* BCG; D, *M. avium*; E, *M. intracellulare*; F, *M. kansasii*; G, *M. marinum*; H, *M. fortuitum*. Molecular weight protein standards: lysozyme (14,400), soybean trypsin inhibitor (21,500) carbonic anhydrase (31,000), ovalbumin (45,000) bovine serum albumin (66,200), phosphorylase B (92,500).

prior to polyacrylamide gel electrophoresis (PAGE) and immunoblotting.

All antigen preparations were stored in small samples at −20°C. Tuberculin purified protein derivative (PPD) RT40 lot 119 was supplied by the Tuberculin Department, Statens Seruminstitut, Copenhagen, Denmark.

MAbs. The MAbs HYT 6, HYT 27, HYT 28, and HÅT 1 were produced as described elsewhere (C. Schou, Z.-L. Yuan, J. Bennedsen, and Å. B. Andersen, Acta Pathol. Microbiol. Immunol. Scand. Sect. C, in press). Briefly, HYT 6, HYT 27, and HÅT 1 were developed from BALB/c mice immunized with H37Rv BPE. HYT 28 was developed from mice immunized with immunoprecipitates excised from agarose gels after crossed immunoelectrophoresis of H37Rv CCF into a second-dimension gel containing polyclonal rabbit antiserum to *M. tuberculosis*. The HÅT 3 antibody was developed after immunizations with H37Rv CCF depleted by immunosorbent chromatography of the antigens corresponding to the HYT 6, HYT 27, and HYT 28 antibodies. Small-scale affinity columns were made by coupling the MAbs to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). H37Rv CCF was passed through the columns, and the effluent was used for immunization.

BALB/c mice were immunized with 100 μg of protein intraperitoneally three times at intervals of 2 weeks and boosted with the same amount of antigen 3 days before removal of the spleen. Screening of supernatants from growing clones was done by ELISA and immunoblotting with H37Rv CCF as the antigen.

Fetal calf serum containing culture supernatants was concentrated by ammonium sulfate precipitation (50%). The precipitates were redissolved and dialyzed in PBS (pH 7.4). Subclass determination was performed by Ouchterlony double diffusion assay with subclass-specific rabbit antisera (Miles Laboratories, Elkhart, Ind.).

SDS-PAGE. PAGE was done in a discontinuous system. The separation gel consisted of 12.5% (wt/vol) acrylamide, 0.4% SDS in 3 M Tris hydrochloride (pH 8.6), and the stacking gel consisted of 4% acrylamide, 0.4% SDS in 1 M Tris hydrochloride (pH 6.8). The cross-linker was *N,N'*-methylenebisacrylamide (2.6%). The samples (usually 50 μg of protein per slot) were applied under reducing conditions. The antigens separated in the polyacrylamide gels were stained with Coomassie brilliant blue or transferred to sheets of nitrocellulose. Protein molecular weight standards were obtained from Bio-Rad Laboratories (Richmond, Calif.).

Immunoblotting. Transfer of antigens from polyacrylamide gels onto nitrocellulose sheets (BA85; 0.45 μm [pore size]; Schleicher & Schuell, Inc., Keene, N.H.) was performed at 10 V/cm for 3 h in 25 mM Tris hydrochloride buffer (pH 9.0) containing 192 mM glycine and 20% (vol/vol) methanol. The nitrocellulose sheets were used immediately or were stored in transfer buffer at 4°C. Before use, the sheets were rinsed thoroughly in distilled water. Reactive sites of the nitrocellulose were blocked by soaking the sheets for 10 min in PBS containing 0.5% Tween 20. Antigens immobilized on nitrocellulose were incubated for 2 h or overnight with MAbs diluted (1:250 or 1:500) in PBS with 0.5 M NaCl and 0.05% Tween 20. Antibodies bound were detected with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulins (P 260; Dakopatts, Glostrup, Denmark) diluted 1:1,000. A substrate-dye solution was made of 3,5,3',5'-tetramethylbenzidine (2.4 mg/ml) dissolved in ethanol-citric acid buffer (pH 5.0; 1:3) containing 0.2% dioctyl sodium sulfosuccinate and 0.06% H_2O_2.

ELISA. ELISA was performed in 96-well polystyrene microtiter plates (Nunc, Roskilde, Denmark) with CCF or BPE as the antigen. CCF-BPE was diluted in sodium carbonate buffer (pH 9.6), and each well was coated with 5 μg

TABLE 1. MAb subclasses and molecular weight estimates of corresponding antigens

MAb	Subclass[a]	Mol wt (10^3) of corresponding antigen[b]
HYT 6	IgG1, K	17–19
HYT 27	IgG1, K	32–33
HYT 28	IgG1, K	38
HÅT 1	IgM	71
HÅT 3	IgG1, K	(smear)

[a] IgG1, Immunoglobulin G1.

[b] Molecular weights were determined in a 5%/15% gradient acrylamide gel with H37Rv CCF as the antigen.

TABLE 2. Interspecies reactivity of the MAbs characterized by immunoblotting

Species	HYT 6[a]				HYT 27[a]				HYT 28[a]				HÅT 1[a]				HÅT 3[a]			
	CCF	BPE	CCWE	WCS[b]	CCF	BPE	CCWE	WCS[b]	CCF	BPE	CCWE	WCS[b]	CCF	BPE	CCWE	WCS[b]	CCF	BPE	CCWE	WCS[b]
M. tuberculosis H37Rv	+	+	+		+	+	+		+	+	+		+	+	+		+	+	+	
M. tuberculosis I95	+	+	+		+	+	+		+	+	+		+	+	+		+	+	+	
M. bovis BCG	+	+	+		+	−	+		+	+	+		+	+	+		+	+	+	
M. avium	+	+	+		+	−	+		−	−	−		+	+	+		(+)	+	+	
M. intracellulare	ND[c]	+	+		ND	+	+		ND	−	−		ND	+	+		ND	+	+	
M. kansasii	+	+	+		+	+	+		−	−	−		+	+	−		+	+	+	
M. marinum	+	+	+		+	+	+		−	−	−		+	+	−		+	+	+	
M. fortuitum	−	−	−		+	+	−		−	−	−		−	−	−		(+)	(+)	+	
N. asteroides				−				−				−				+				+
R. erythropolis				−				−				−				−				+
E. coli				−				−				−				−				−

[a] +, (+), and − indicate clear, weak, and no reaction, respectively.
[b] WCS, Whole-cell sonic extract.
[c] ND, Not done.

of protein. The antigen was allowed to bind overnight at 4°C. For blocking of additional reactive sites in the wells and for dilution of the antibodies, PBS containing 1% bovine serum albumin and 1% Triton X-100 was used. The MAbs were allowed to bind for 2 h at room temperature. Horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin (1:1,000) was added for 1 hour. The substrate-dye solution (*O*-phenylenediamine [0.4 mg/ml] and H_2O_2 [0.04%]) was allowed to react for 10 min, and the reaction was stopped by adding 2 N H_2SO_4. Optical density was measured at 492 nm.

As a negative control, fetal calf serum containing medium concentrated in the same way and to the same extent as the culture supernatants was used. Wells showing an optical density exceeding or equal to twice the mean background level were considered positive. The MAbs were titrated in an ELISA with *M. tuberculosis* H37Rv CCF as the antigen. A dilution (1:40 to 1:160) giving an optical density at the maximum plateau of the titration curve was used as the working dilution in the interspecies analysis. Because HYT 28 and HÅT 1 did not exhibit a plateau and only reacted weakly in ELISA, they were tested in dilutions of 1:2 to 1:8.

Lectin chromatography. CCF (7.5 mg) (*M. tuberculosis* H37Rv, *M. tuberculosis* I95) was passed through a column consisting of 20 ml of Sepharose-conjugated concanavalin A (Pharmacia). Elution was done with 0.5 M α-methyl-D-glucoside (Sigma Chemical Co., St. Louis, Mo.). The effluent and eluted fractions were tested in a dot blot assay by applying 10 μl per dot. Incubation with MAbs and staining were done as described for the immunoblotting procedure. The eluate could be stained by the protein stain amido black.

Protease treatment. H37Rv CCF (100 μg) was digested with the enzyme pronase (Boehringer) for 45 min at 37°C. The reaction was stopped by boiling the samples in the presence of SDS and β-mercaptoethanol. The digested material was analyzed by immunoblotting (data not shown).

TABLE 3. Interspecies reactivity of the MAbs characterized by ELISA[a]

Species	HYT 6		HYT 28		HÅT 1		HÅT 3	
	CCF	BPE	CCF	BPE	CCF	BPE	CCF	BPE
M. tuberculosis H37Rv	+[b]	+	+/−[c]	−	+	+/−	+	+
M. tuberculosis I95	+	+	+/−	−	+	+/−	+	+
M. bovis BCG	+/−	+/−	−	−	+	+/−	+	+/−
M. avium	+/−	+/−	−	−	+/−	+/−	+/−	+
M. intracellulare	ND[d]	−	ND	−	ND	+/−	ND	+
M. kansasii	+	+	−	−	+	+/−	+	+
M. marinum	+	+	−	−	+	+/−	+	+
M. fortuitum	−	−	−	−	−	−	−	+/−

[a] Mean results from five experiments.
[b] +, Optical densities exceeding or equal to twice the mean background level.
[c] +/−, Optical densities close to cutoff values, giving positive results in some experiments and negative results in others.
[d] ND, Not done.

RESULTS

Three different preparations of mycobacterial antigens were prepared: CCF, BPE, and CCWE. *M. avium* yielded little and *M. intracellulare* no detectable antigens in CCF. Protein components of the various preparations were resolved by PAGE, and the distinct patterns obtained by staining the gels with Coomassie brilliant blue are shown in Fig. 1. The PAGE patterns of the CCWEs appeared as smears (data not shown).

The subclasses of the MAbs and the molecular weights of antigens from *M. tuberculosis* CCF to which the MAbs bind are shown in Table 1. CCF, BPE, and CCWE of each species

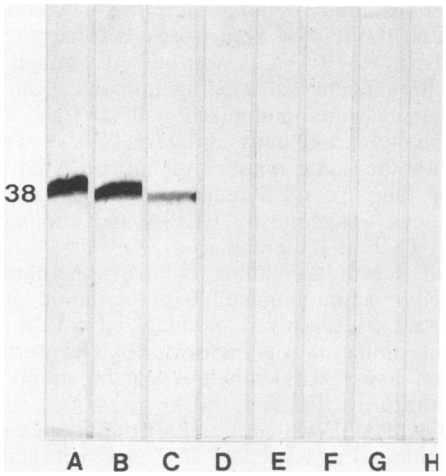

FIG. 2. Immunoblotting analysis. HYT 28 incubated with CCWEs from (lanes): A, *M. tuberculosis* H37Rv; B, *M. tuberculosis* I95; C, *M. bovis* BCG; D, *M. avium*; E, *M. intracellulare*; F, *M. kansasii*; G, *M. marinum*; and H, *M. fortuitum*. Molecular weight in thousands is indicated on the left.

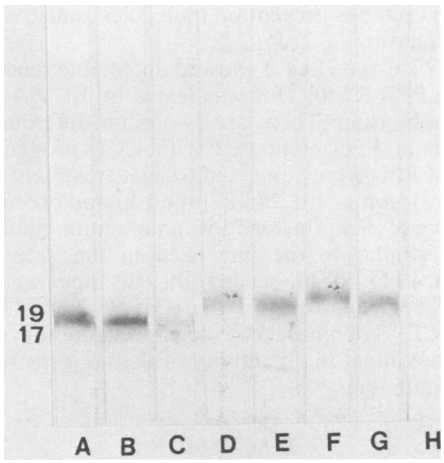

FIG. 3. Immunoblotting analysis; HYT 6 incubated with BPEs from (lanes): A, *M. tuberculosis* H37Rv; B, *M. tuberculosis* I95; C, *M. bovis* BCG; D, *M. avium*; E, *M. intracellulare*; F, *M. kansasii*; G, *M. marinum*; and H, *M. fortuitum*. Molecular weight in thousands is indicated on the left.

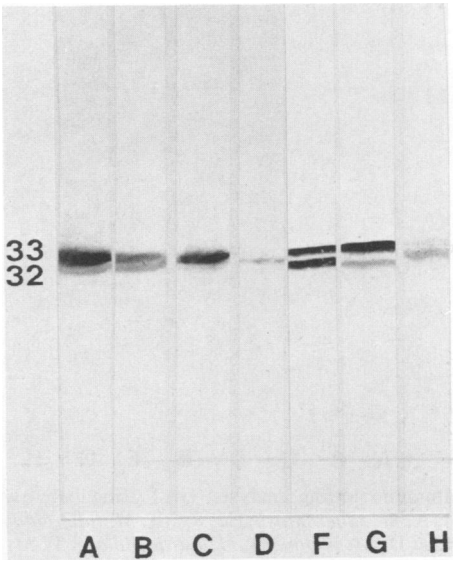

FIG. 5. Immunoblotting analysis; HYT 27 incubated with CCFs from (lanes): A, *M. tuberculosis* H37Rv; B, *M. tuberculosis* I95; C, *M. bovis* BCG; D, *M. avium*; F, *M. kansasii*; G, *M. marinum*; and H, *M. fortuitum*. Molecular weight in thousands is indicated on the left.

were used as antigens in immunoblotting experiments, and the results are summarized in Table 2. Qualitative differences in the reaction patterns, depending on which antigen preparations they were tested with, were observed with the MAbs HYT 27 and HÅT 3 (see Fig. 4, 5, and 7). Only CCF and BPE preparations were applied in the ELISA. ELISA results are shown in Table 3. For the sake of clarity, reactions were divided into three groups only (+, +/−, and −). Reactions to CCF were generally stronger than reactions to BPE.

MAb HYT 28 reacted with a 38-kilodalton (kDa) molecule which was found solely in *M. tuberculosis* and *M. bovis* BCG (Fig. 2). Only very weak reactions were seen by ELISA.

Immunoblotting with HYT 6 produced reactions with all mycobacterial species examined except *M. fortuitum* (Fig. 3). ELISA reactions with HYT 6 were generally very strong except with antigens of *M. bovis* BCG, *M. avium*, and *M. intracellulare*. HYT 6-binding epitopes were, however, clearly demonstrated also in these species by immunoblotting (Fig. 3).

HYT 27 recognized an epitope present in all the mycobacterial species examined (Fig. 4 and 5). No reactivity to *N. asteroides*, *R. erythropolis*, or *E. coli* could be dem-

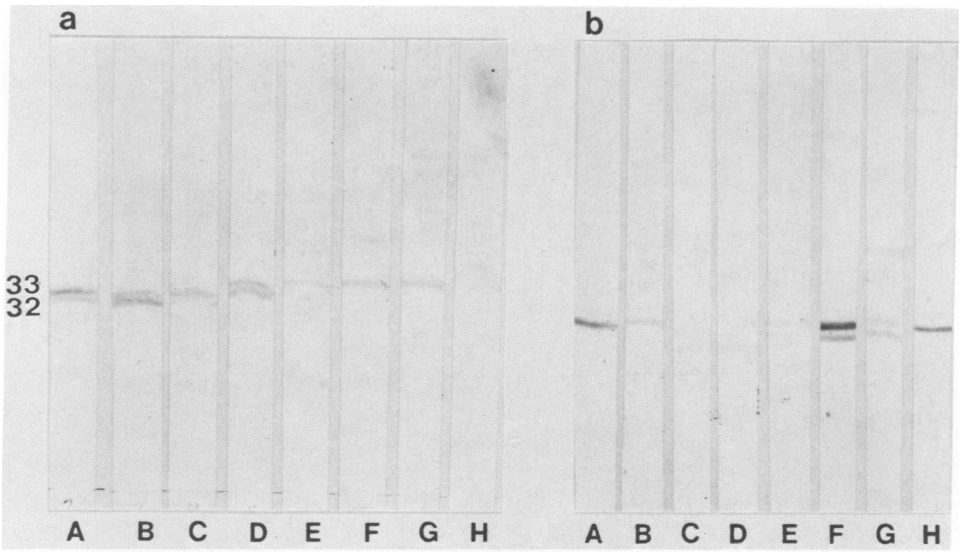

FIG. 4. Immunoblotting analyses; HYT 27 incubated with CCWEs (panel a) and BPEs (panel b) from (lanes): A, *M. tuberculosis* H37Rv; B, *M. tuberculosis* I95; C, *M. bovis* BCG; D, *M. avium*; E, *M. intracellulare*; F, *M. kansasii*; G, *M. marinum*; and H, *M. fortuitum*. Molecular weight in thousands is indicated on the left.

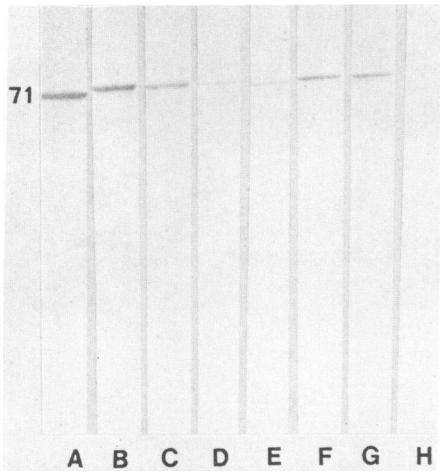

FIG. 6. Immunoblotting analysis; HÅT 1 incubated with BPEs from (lanes): A, *M. tuberculosis* H37Rv; B, *M. tuberculosis* I95; C, *M. bovis* BCG; D, *M. avium*; E, *M. intracellulare*; F, *M. kansasii*; G, *M. marinum*; and H, *M. fortuitum*. Molecular weight in thousands is indicated on the left.

onstrated. HYT 27 appeared to cross-react with bovine serum albumin and was therefore not tested by ELISA.

Immunoblots with HÅT 1 showed reactions with an epitope on a 71-kDa molecule present in all the mycobacterial species tested except *M. fortuitum* (Fig. 6). HÅT 1 also bound to a molecule of similar molecular weight in *N. asteroides*.

HÅT 3 bound to an epitope present on a number of molecules (among these a 71-kDa molecule) which were found in all mycobacterial species tested (Fig. 7). HÅT 3 also reacted with a 71-kDa molecule present in *N. asteroides* as well as *R. erythropolis*. Binding to *E. coli* could not be demonstrated with any of the MAbs.

The antigens recognized by HYT 6 and HÅT 1 were bound by Sepharose-coupled concanavalin A and could be specifically eluted with α-methyl-D-glucoside. All the MAbs reacted with epitopes present on molecules sensitive to digestion by pronase.

Only HYT 6 and HÅT 3 showed detectable reactions with tuberculin PPD RT40. This was tested by ELISA as well as by immunoblotting. There are two main differences in the production of PPD compared with CCF production. The antigens of PPD were subjected to heat treatment (steaming at 1.3 atm [1 atm = 101.29 kPa] for 1 h) and precipitated by trichloroacetic acid instead of ammonium sulfate. Heat treatment similar to the one used in the preparation of tuberculin PPD RT40 caused the disappearance of the epitopes recognized by HYT 27, HYT 28, and HÅT 1 from H37Rv CCF. Trichloroacetic acid precipitation of H37Rv CCF did not affect the reactivity of the antigens recognized by these antibodies.

DISCUSSION

Five murine MAbs to *M. tuberculosis* were evaluated with respect to their cross-reactivity with other mycobacterial species by ELISA and immunoblotting. Each MAb demonstrated a unique reactivity pattern, ranging from one MAb (HYT 28), which reacted solely with *M. tuberculosis* and *M. bovis* BCG, to another MAb (i.e., HÅT 3), which reacted with all the mycobacterial species examined, as well as *N. asteroides* and *R. erythropolis*. None of the MAbs reacted with *E. coli*. These characteristics may prove useful in the identification and typing of mycobacteria. Quantitative preparation of the antigens corresponding to these MAbs by immunosorbent chromatography is now possible and is being carried out in our laboratory. This will enable us to examine the biological roles of the antigens. Our knowledge of the chemical composition of these antigens is limited to the finding that the epitopes recognized by the MAbs are located on molecules sensitive to pronase. This indicates that the antigens are, at least in part, polypeptides. Two of the MAbs, HYT 6 and HÅT 1, reacted with molecules possessing concanavalin A-binding moieties, suggesting that these antigens may be glycoproteins. Daniel et al. (2) have previously described four MAbs reacting with *M. tuberculosis* antigen 5. This antigen has been characterized as a

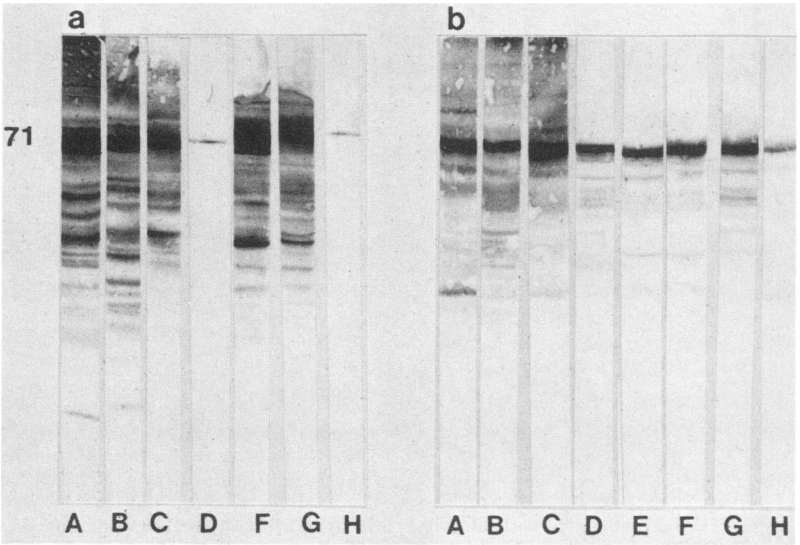

FIG. 7. Immunoblotting analyses; HÅT 3 incubated with CCFs (panel a) and BPEs (panel b) from (lanes): A, *M. tuberculosis* H37Rv; B, *M. tuberculosis* I95; C, *M. bovis* BCG; D, *M. avium*; E, *M. intracellulare*; F, *M. kansasii*; G, *M. marinum*; and H, *M. fortuitum*. Molecular weight in thousands is indicated on the left.

distinct precipitin arc by immunoelectrophoresis and is known to have affinity for concanavalin A.

Considering the extensive use of tuberculin PPD, it was of interest to determine whether it contained any of the antigens defined by our MAbs. Only two of the five epitopes could be demonstrated.

For interspecies analysis, and ELISA is a convenient method. However, because of the arbitrarily chosen cutoff values, the validity of the results may be questioned. Some workers consider optical density values exceeding 0.1 to 0.2 positive (13); others rely on visual evaluation (6, 8). Coates et al. (1) developed a modified radioimmunoassay in which maximum binding capacity of the MAb was determined and the interspecies analysis was performed as a competition assay. In the present study, immunoblotting analyses gave qualitative results, which facilitated evaluation of weak or questionable ELISA reactions. Apart from the problem of the cutoff level, the ELISA was complicated by the problem that not all mycobacterial antigens bound equally well to polystyrene. This was most clearly observed with HYT 28, which reacted only weakly in the ELISA but was evidently positive in the immunoblotting assay. This observation might, however, also be explained by demasking of the epitope subsequent to SDS treatment of the antigen.

From the World Health Organization workshop to compare MAbs against *M. tuberculosis* and *M. leprae* (June 1985, Geneva, Switzerland), it has become evident that HYT 28 is similar to TB72, a MAb developed by Coates et al. (1). Both MAbs are of the same subclass and react with a 38-kDa molecule which we found only in *M. tuberculosis* and *M. bovis* BCG. The use of TB72, initially reported to be specific for *M. tuberculosis* (1), was evaluated by Ivanyi et al. (5) in a serological competition assay as a possible diagnostic test for tuberculosis. The present study shows that *M. bovis* BCG also contains the antigen and that, therefore, the specificity of the test in a BCG-vaccinated population must be studied thoroughly. MAbs defining epitopes present exclusively on *M. tuberculosis* have, so far, not been produced.

ACKNOWLEDGMENT

This work was supported by the Danish National Association against Tuberculosis and Lung Disease.

LITERATURE CITED

1. **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.
2. **Daniel, T. M., N. J. Gonchoroff, J. A. Katzmann, and G. R. Olds.** 1984. Specificity of *Mycobacterium tuberculosis* antigen 5 determined with mouse monoclonal antibodies. Infect. Immun. **45**:52–55.
3. **Daniel, T. M., and B. W. Janicki.** 1978. Mycobacterial antigens: a review of their isolation, chemistry, and immunological properties. Microbiol. Rev. **42**:84–113.
4. **Gillis, T. P., and T. M. Buchanan.** 1982. Production and partial characterization of monoclonal antibodies to *Mycobacterium leprae*. Infect. Immun. **37**:172–178.
5. **Ivanyi, J., E. Krambovitis, and M. Keen.** 1983. Evaluation of a monoclonal antibody (TB72) based serological test for tuberculosis. Clin. Exp. Immunol. **54**:337–345.
6. **Kolk, A. H. J., M. L. Ho, P. R. Klatser, T. A. Eggelte, S. Kuijper, S. De Jonge, and J. van Leeuwen.** 1984. Production and characterization of monoclonal antibodies to *Mycobacterium tuberculosis*, *M. bovis* (BCG) and *M. leprae*. Clin. Exp. Immunol. **58**:511–521.
7. **Markwell, M. A. K., S. M. Haas, L. L. Bieber, and N. E. Tolbert.** 1978. A Modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. **87**:206–210.
8. **Matthews, R., A. Scoging, and A. D. M. Rees.** 1985. Mycobacterial antigen-specific human T-cell clones secreting macrophage activating factors. Immunology **54**:17–23.
9. **Minden, P., P. J. Kelleher, J. H. Freed, L. D. Nielsen, P. J. Brennan, L. McPheron, and J. K. McClatchy.** 1984. Immunological evaluation of a component isolated from *Mycobacterium bovis* BCG with a monoclonal antibody to *M. bovis* BCG. Infect. Immun. **46**:519–525.
10. **Young, D. B., S. R. Khanolkar, L. L. Barg, and T. M. Buchanan.** 1984. Generation and characterization of monoclonal antibodies to the phenolic glycolipid of *Mycobacterium leprae*. Infect. Immun. **43**:183–188.