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# Purification of the 65 kD Protein from *Mycobacterium gordonae* and Use in Skin Test Response to *Mycobacterium leprae*[1,2]

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The study of protein antigens of *Mycobacterium leprae* is based on two generally accepted concepts of immunobiology: a) that proteins are generally potent immunogens capable of stimulating both B cells, to produce antibodies, and T cells, to mediate various effector systems, commonly referred to as cell-mediated immunity (CMI); and b) that resistance to infection by intracellular parasites is conferred by CMI. Protein antigens of *M. leprae* have been the focus of intensive study recently with the application of new molecular technologies, including DNA cloning and monoclonal antibody (Mab) production. These methodologies have provided the tools to study proteins of *M. leprae* in great detail and should provide purified, well-characterized antigenic molecules with which to dissect the immune response to *M. leprae*.

Recent discoveries using Mabs have established that certain proteins of *M. leprae* or epitopes on these proteins constitute species-specific or crossreactive antigenic determinants of *M. leprae* ([7]). Some of these epitopes have been shown to be immunogenic in man based on the detection of antibody to a given epitope in the sera of leprosy patients ([9, 25]) or by their ability to stimulate the proliferation of cloned T lymphocytes either from *M. leprae*-immunized humans ([20]) or tuberculoid leprosy patients ([21]). The cell wall-associated protein (CWP) antigen of *M. leprae* ($M_r$ = 65,000) is immunogenic in man and carries one species-specific epitope and at least four crossreactive epitopes ([9, 10]). We have shown that antibodies are produced in leprosy patients to both the crossreactive and species-specific determinants of the CWP of *M. leprae*. In contrast to serologic reactivity of the CWP of *M. leprae*, nothing is known of the potential reactivity of this protein with lymphocytes involved in cell-mediated reactions to *M. leprae* or to other mycobacteria. While the amino-acid sequence of the 65 kD protein has been determined ([17]), neither native nor recombinant 65 kD protein has been purified to homogeneity for further study. Accordingly, we have purified the homologous crossreactive protein of *M. gordonae* ($M_r$ = 65,000) which shares at least three antigenic determinants with the 65 kD CWP of *M. leprae*, using a monoclonal antibody affinity purification scheme. Conditions were established which produced optimal purification of this protein from crude protein mixtures. With the purified protein, we then tested the ability of common epitopes of the 65 kD molecule to elicit a delayed-type hypersensitivity (DTH) response in guinea pigs as a measure of T-cell responsiveness to this protein. Our results show that highly purified 65 kD from *M. gordonae* can be obtained by affinity chromatography and that common epitopes do elicit a strong DTH response in guinea pigs sensitized with *M. gordonae*, *M. bovis* (BCG), or *M. leprae*.

## MATERIALS AND METHODS

**Growth and extraction of *M. gordonae*.** *M. gordonae* ATCC 14470 were grown in Dubos media without Tween 80 (Difco Laboratories, Detroit, Michigan, U.S.A.) supplemented with 0.75% glucose and 0.2% pyruvic acid. Cultures were grown in 2-liter amounts with constant shaking at 37°C for 5–7 days in 4-liter flasks. Cells were harvested and concentrated to one sixth their

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original volume by tangential flow filtration with a Pellicon cassette system (Millipore Corp., Bedford, Massachusetts, U.S.A.). Cells were washed extensively in the same apparatus with 0.01 M phosphate-buffered normal saline (PBS) containing 0.05% Tween 80. Cells were harvested from the concentrate by centrifugation at 10,000 × $g$ × 15 min, and stored as a pellet at −70°C.

A crude extract of *M. gordonae* was prepared from disrupted microorganisms following sonication. Briefly, 2 g (wet weight) of *M. gordonae* was resuspended in 40 ml of the sonication buffer, which consisted of PBS containing 0.05% Tween 80, 1 mM benzamidine-HCl at pH 7.0. This suspension was sonicated for 45 min with short bursts of energy delivered intermittently such that the temperature of the sonication mixture did not exceed 8°C. The sonicated mixture was centrifuged at 30,000 × $g$ × 20 min. The resultant supernatant fraction was centrifuged at 100,000 × $g$ × 90 min to yield a supernatant fraction which was filtered through a 0.45 $\mu$ filter resulting in 40 ml of the final extract (*M. gordonae* 100ks) with a protein concentration of 2 mg/ml.

Irradiated, armadillo-derived *M. leprae* used for guinea pig immunizations were obtained from Dr. Patrick Brennan through support from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Contract no. 1 AI52582. *M. bovis* (BCG) Pasteur strain was a gift from Dr. J. L. Krahenbuhl.

**Mab IIC8 affinity matrix.** Mab IIC8 is a murine Mab produced by immunization of BALB/c mice with *M. leprae* as described previously ([8]). Mab IIC8 recognizes a cross-reactive epitope on the CWP of *M. leprae* which has been designated the CWP$^c$ epitope of the 65 kD protein ([10]). This epitope has been detected in extracts of *M. leprae, M. gordonae, M. bovis* BCG, *M. gastri, M. flavescens, M. triviale, M. marinum,* and *M. smegmatis* ([8]). Mab IIC8 was purified from ascites on a protein A (Pharmacia Fine Chemicals, Piscataway, New Jersey, U.S.A.) affinity column. Purified Mab IIC8 was eluted from the protein A column at pH 3.5 in a 0.1 M citrate buffer, and immediately neutralized with the addition of 1.0 M Tris-HCl buffer, pH 9.0. Column fractions containing purified IIC8 were pooled, dialyzed against the coupling buffer (0.1 M bicarbonate buffer, pH 8.2, containing 0.5 M NaCl), and concentrated by pressure filtration (Amicon Corp., Canvers, Massachusetts, U.S.A.) on a PM-30 membrane to a protein concentration of approximately 3.0 mg/ml. Twenty ml of this material was reacted with 3 g (dry weight) of cyanogen bromide-activated Sepharose 4B (Pharmacia) as described by the manufacturer. Under these conditions, approximately 90% of Mab IIC8 was bound to the Sepharose 4B matrix.

**Purification of *M. gordonae* 65 kD protein on IIC8 affinity column.** The IIC8 affinity matrix was equilibrated in charging buffer (0.05 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl and 0.05% Triton X-100) prior to the addition of the *M. gordonae* 100ks antigen extract. Twenty ml of *M. gordonae* 100ks was added to the IIC8 affinity matrix and held for 2 hr at 4°C with gentle mixing. The mixture was applied to a column (9 × 1.8 cm), and the elution of unbound components initiated with charging buffer. Five ml fractions were collected, and the absorbance of individual fractions was monitored at 280 nm. Subsequent elution conditions consisted of wash-I buffer (50 ml) containing 0.05 M Tris-HCl, pH 8.0, 0.45 M NaCl, and 0.05% Triton X-100 followed by wash-II buffer (50 ml), consisting of wash-I buffer minus Triton X-100. Some column runs were then developed with a decreasing pH step gradient to include pH 6.8, 5.7, 5.0, 4.0, and 3.0. Other columns were developed with only pH 5.7, 4.2, and 2.8 after wash-I and wash-II buffers. All low pH elution buffers consisted of 0.03 M barbital-acetate containing 2.4 M urea, except the pH 2.8 buffer which was 1.0 M acetic acid with 2.0 M urea. Fractions representing each elution condition were pooled, dialyzed extensively against deionized $H_2O$, and lyophilized. The dried material was resuspended in 2 ml of sterile PBS and passed through a 0.22 $\mu$ filter. Fractions representing the first peak (Peak I) or unbound material, and the first wash (wash-I), were pooled separately, and concentrated to 10 ml by pressure filtration using PM-10 membranes (Amicon).

**Polyacrylamide gel electrophoresis and immunoblot.** Polyacrylamide gel electrophoresis (PAGE) was performed by the method of Laemmli ([13]). Conditions for

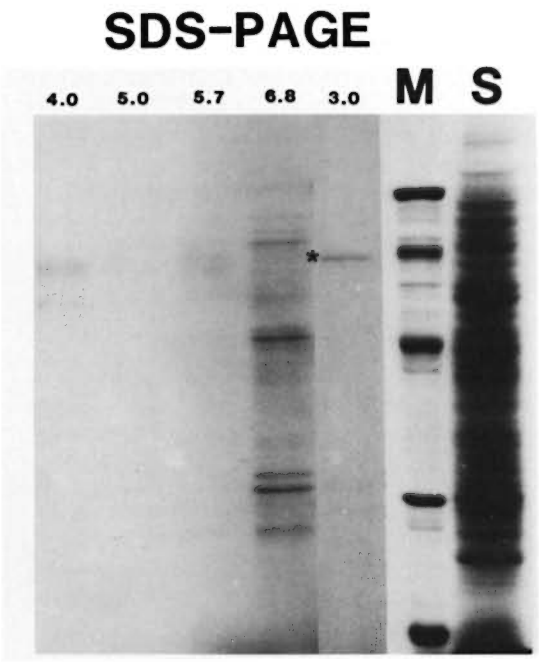


FIG. 1. SDS-PAGE analysis of *M. gordonae* 100ks fractions from a Mab IIC8 affinity column. Lanes 3.0, 4.0, 5.0, 5.7, 6.8 represent the fractions eluted from the column at those pH values. Protein concentrations for lanes 3.0 and 4.0 were 50 μg/ml; for lanes 5.0 and 5.7, 20 μg/ml; for lane 6.8, 200 μg/ml; and for S (*M. gordonae* 100ks), 2.0 mg/ml. The marker (*) indicates $M_r$ = 65,000. The molecular weight markers (lane M) were: phosphorylase b = 92,500; bovine serum albumin = 66,200; ovalbumin = 45,000; carbonic anhydrase = 31,000; and soybean trypsin inhibitor = 21,500.

polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) and 2-mercaptoethanol and immunoblotting were the same as those described previously ([8]). Samples resolved by SDS-PAGE for subsequent staining or immunoblotting were solubilized by heating for 3 min at 100°C in an equal volume of sample buffer containing 0.2% SDS, 0.286 M 2-mercaptoethanol, and 20% glycerol in 100 mM Tris hydrochloride, pH 6.8. Gels were stained with silver as described by manufacturer (Bio-Rad Laboratories, Richmond, California, U.S.A.). Protein determinations were performed by the method of Lowry, *et al.* [15].

**Immunization and skin testing of guinea pigs.** Two-month-old, female Hartley guinea pigs were immunized with one of the following preparations: Group I = Freund's incomplete adjuvant (FIA) mixed with an equal volume of sterile normal saline, 0.2 ml intramuscular; Group II = *M. gordonae* 100ks (3.0 mg/ml, protein) in FIA, 0.2 ml intramuscular; Group III = autoclaved *M. leprae* (1.0 mg/ml, dry weight) in PBS given intradermally at five sites, 0.1 ml per site; Group IV = heat-killed *M. bovis* BCG (3.5 mg/ml, wet weight) in FIA, 0.2 ml intramuscular; Group V = autoclaved *M. gordonae* (1 mg/ml, dry weight) in PBS given intradermally at five sites, 0.1 ml per site. Each group consisted of four guinea pigs which were housed in pairs.

Thirty days after immunization and 24 hr prior to skin testing, a skin-test site (8 × 5 cm) was prepared by hair removal with clippers and a depilatory cream on the abdomen of each animal. Each animal was challenged with an intradermal injection of 0.1 ml of sterile saline and 25 μg of affinity-purified protein from *M. gordonae* ($M_r$ = 65,000) at separate sites. Forty-eight hr after skin testing, each site was measured for induration as a double skin-fold thickness in millimeters with dial gauge calipers (Schnell-täster system; Kropelin, West Germany). Each skin-test site was then excised, fixed in 10% buffered Formalin, and processed for paraffin sections. Five-μm sections were cut, stained with hematoxylin and eosin (H&E), and examined by light microscopy.

## RESULTS

**Purification of 65 kD protein of *M. gordonae*.** Protein staining of SDS-PAGE profiles of various fractions obtained from the Mab IIC8 affinity column are shown in Figure 1. Numerous proteins were seen in *M. gordonae* 100ks (lane S), one of which constituted the 65 kD protein antigen of *M. gordonae* recognized by immunoblotting with Mab IIC8 (Fig. 2, lane S). Peak I, which represents the material not bound by the affinity matrix, showed a protein-staining profile not unlike *M. gordonae* 100ks (data not shown). Immunoblots of Peak I contained very small amounts of 65 kD protein (Fig. 2, lane PI). As the pH of column buffer was reduced to 6.8 in the presence of 2.4 M urea, numerous proteins were released (Fig. 1, lane 6.8) but the 65 kD protein was not detected by immunoblotting (Fig. 2, lane 6.8). Similarly, immunoblots of fractions 5.7

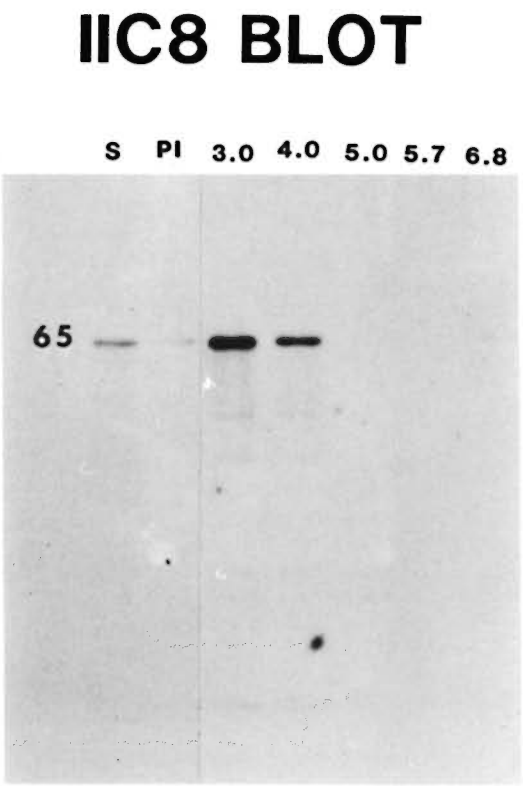


FIG. 2. Immunoblot analysis of SDS-PAGE profiles of *M. gordonae* 100ks fractions eluted from a Mab IIC8 affinity column. Lanes are the same as in Figure 1, except that the protein concentrations were 50 μg/ml for 3.0, 4.0, 5.0, 5.7, and 6.8. Peak I (PI) and *M. gordonae* 100ks (S) were 2 mg/ml. The immunoblot was developed with Mab IIC8 and $^{125}$I-labeled *Staphylococcus aureus* protein A. The marker (*) indicates $M_r$ = 65,000.

and 5.0 were negative for the 65 kD protein (Fig. 2).

The pH 4.0 fraction contained a diffuse protein-staining band(s) at $M_r$ = 65,000 on silver-stained gels (Fig. 1, lane 4.0), and a strong band of immunoreactivity at the same molecular weight on the immunoblot (Fig. 2, lane 4.0). Similarly, the pH 2.8 fraction contained a single polypeptide ($M_r$ = 65,000) by protein staining (Fig. 1, lane 3.0) and a very strong band of immunoreactivity by immunoblotting (Fig. 2, lane 3.0).

Affinity fractions pH 3.0 and pH 4.0 were pooled and concentrated to yield a protein concentration of 250 μg/ml. Quantitation of simple sugars, oligosaccharides, polysaccharides, and their derivatives in the affinity preparation was by the phenol sulfuric acid test as described by Dubois, *et al.* [6] with glucose as a standard. The source material (*M. gordonae* 100ks) contained 1.0 mg/ml total hexoses and the 65 kD affinity fraction was below detectable limits of the assay (<10 μg/ml).

THE TABLE. *Skin test results.*

| Group[a] | Skin-fold thickness[b] | |
|---|---|---|
| | PBS | 65 kD |
| I. Freund's | 20 ± 3 | 18 ± 3 |
| II. *M. gordonae* 100ks | 21 ± 3 | 30 ± 7 |
| III. *M. leprae* | 20 ± 3 | 25 ± 3[c] |
| IV. *M. bovis* (BCG) | 21 ± 5 | 27 ± 4 |
| V. *M. gordonae* | 20 ± 2 | 34 ± 6[d] |

[a] See Materials and Methods for group designations.
[b] Mean and standard deviation in tenths of a millimeter (N = 4/group).
[c] Significantly different from within-group PBS skin test, induration at $p < 0.01$ by paired-*t* test.
[d] Significantly different from within-group PBS skin test, induration at $p < 0.05$ by paired-*t* test.

**Gross and microscopic observations of skin tests.** Gross changes at the skin-test sites at 48 hr were measured by double skin-fold thickness in tenths of a millimeter and are summarized in The Table. Saline skin-test sites were uniformly nonreactive with a mean skin-fold thickness of 20.4 ± 0.5. In contrast, induration and erythema were observed at the 65 kD skin-test site on all animals except those injected with FIA. Histopathological observations confirmed the gross appearance of the skin-test sites. Saline skin-test sites from all animals and 65 kD test sites from guinea pigs injected with FIA showed a few scattered mononuclear cells in the dermis which was consistent with a normal appearance (Fig. 3a). In contrast, the 65 kD skin-test sites from *M. gordonae* (Fig. 3 b and e), *M. bovis* BCG (Fig. 3d), and *M. leprae* (Fig. 3c) immunized animals showed an intense inflammatory reaction. The dermis and the subcutaneous tissue were densely infiltrated with numerous macrophages, lymphocytes, and scattered neutrophils. Well marked edema and increased vascularity were also present. In a few areas, the inflammation extended deeper into the muscle tissue.

## DISCUSSION

Development and utilization of Mab technology has had a major impact on many

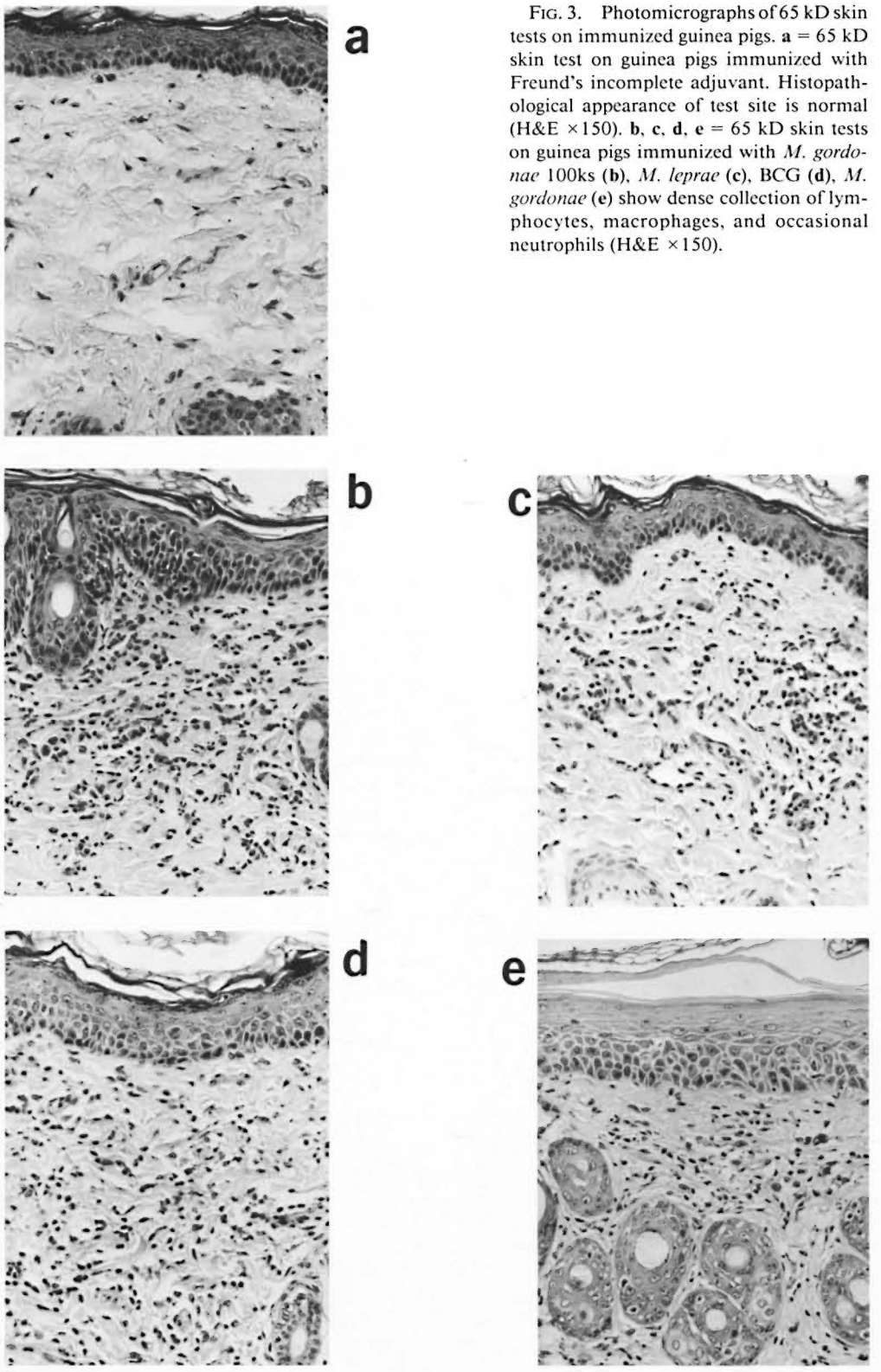


FIG. 3. Photomicrographs of 65 kD skin tests on immunized guinea pigs. **a** = 65 kD skin test on guinea pigs immunized with Freund's incomplete adjuvant. Histopathological appearance of test site is normal (H&E ×150). **b**, **c**, **d**, **e** = 65 kD skin tests on guinea pigs immunized with *M. gordonae* 100ks (**b**), *M. leprae* (**c**), BCG (**d**), *M. gordonae* (**e**) show dense collection of lymphocytes, macrophages, and occasional neutrophils (H&E ×150).

fields, including immunochemical studies of bacterial antigens. In mycobacterial research and particularly in *M. leprae* immunochemical studies, Mab have been invaluable for protein antigenic analyses ([1, 8, 10–12]). We have produced and characterized Mab to *M. leprae* to provide molecular probes with which to purify and to characterize the eliciting antigen(s). We reported previously that Mab IIC8 reacted with a multideterminant cell wall-associated protein ($M_r$ = 65,000) of *M. leprae* ([10]). Some of these determinants were shared with cultivable species of mycobacteria, including *M. gordonae.* We have taken advantage of the crossreactivity of Mab IIC8 to prepare an affinity column to purify the 65 kD protein from *M. gordonae.* The purified protein was then tested for its ability to elicit a DTH reaction in the skin of guinea pigs immunized with *M. gordonae, M. bovis* (BCG), or *M. leprae.*

Simplified elution schemes (e.g., absence of detergent, urea, or NaCl) used with the affinity column did not yield highly purified preparations of the 65 kD protein. In fact, the majority of "contaminating" proteins which eluted at or near neutrality did so only in the presence of urea, a moderately strong denaturing agent. The protein yield of affinity fractions 4.0 and 3.0 were 100 μg and 50 μg, respectively, when 120 mg *M. gordonae* 100ks were applied to the column. These two fractions represented approximately 0.1% of the total protein applied to the column. This yield was not unexpected since the protein-staining profile of *M. gordonae* 100ks indicated only a minor protein band at 65 kD on SDS-PAGE. Preparative scale affinity columns, prepared as described above, have demonstrated an effective lifetime of four chromatographic cycles. Beyond four cycles, the purity and yield of the low pH affinity fractions were diminished.

The animal studies were undertaken to define in greater detail the immunological activity of the 65 kD protein of *M. gordonae* and, by inference, that of *M. leprae,* and to establish its reactivity as a skin-test antigen. This protein was studied because of its established crossreactivity with the 65 kD cell wall-associated protein of *M. leprae* ([10]) and because affinity chromatography techniques, as described above, provided relatively large amounts of this protein for study. Based on the predicted yield of 65 kD protein from available *M. leprae,* direct analysis of the purified native *M. leprae* 65 kD protein was impractical. This limitation should be overcome in the future with the recent preparation of an *M. leprae* genomic library in the lambda gt11 expression vector ([26]). Manipulation of this and related recombinant DNA systems should provide suitable amounts of protein antigens of *M. leprae,* including the 65 kD protein for detailed study.

The strong skin-test responses elicited by the 65 kD protein in these experiments provided evidence that certain antigenic epitopes of this protein were capable of reacting with lymphocytes involved in the DTH response. While the DTH as measured by skinfold thickness was greatest in the animals immunized with autoclaved *M. gordonae,* a significant reaction was detected in the *M. leprae*-immunized group as well. DTH responses measured similarly in the groups immunized with BCG or *M. gordonae* 100ks were visible but not statistically significant. In contrast, microscopic examination of cellular infiltrates at the 65 kD protein sites in all groups, except Freund's, clearly demonstrated a potent DTH reaction to the 65 kD protein. Together, these data showed that at least some of the epitopes responsible for the DTH response were shared with the crossreactive 65 kD proteins of *M. leprae, M. bovis* (BCG), and *M. gordonae,* suggesting that similar cellular reactivity may occur in humans infected with *M. leprae* or any other mycobacteria expressing this antigen. Furthermore, it should be possible to determine the relationship between epitopes reactive with antibody and those recognized by T cells through *in vitro* studies of T-cell responses to the 65 kD protein in the presence and absence of monoclonals with defined specificities.

Virtually all of the previous work in the area of CMI to *M. leprae* has relied on crude multicomponent antigens to test for sensitization by skin testing. For example, Mehra and Bloom demonstrated strong DTH responses in guinea pigs immunized with *M. leprae* when skin testing was performed either with a water-soluble extract of *M.*

*leprae* or intact *M. leprae* ([16]). Similarly, elicitation of DTH responses in various mouse models has been with sonicates of mycobacteria ([24]), whole bacilli ([14]), and crude soluble preparations such as PPD ([2]).

The most widely used skin-test reagents capable of indicating previous exposure to mycobacterial pathogens are the purified protein derivatives (PPD) of *M. tuberculosis* ([22]) and the atypical mycobacteria which cause human infections. It is well established that PPD is a crude preparation and exhibits a limited degree of species specificity ([5]). While highly purified components from PPD ([4]) and BCG ([19]) have been isolated and studied for DTH reactivity, none has supplanted PPD for use as a skin-test reagent. Two less-well-established soluble skin-test antigen preparations from *M. leprae* have been reported, but the specificity, purity, and reliability of these preparations remain questionable ([3, 23]). The 65 kD protein of *M. gordonae* is highly purified but also nonspecific, and thereby limited in its applicability as a skin-test reagent. In contrast, the 65 kD protein of *M. leprae* carries at least one species-specific epitope in addition to at least four crossreactive epitopes. We have produced recombinant 65 kD of *M. leprae* in a lysogenic strain of *Escherichia coli* constructed from a clone derived from the *M. leprae* DNA library present in the lambda gt11 vector ([26]). The recombinant 65 kD protein retains both crossreactive and species-specific monoclonal epitopes when prepared in this manner. Using a recombinant DNA approach for the production of *M. leprae* proteins, it should be possible to isolate the 65 kD protein or a peptide(s) with species specificity which could be tested for DTH responses both in *M. leprae*-infected animals and man. A similar approach using a peptide from *M. tuberculosis* has shown promise as a skin-test reagent ([18]).

In the event that an *M. leprae*-specific skin test reagent is found, it may be possible to detect prior exposure to the leprosy bacilli at a preclinical stage of the disease. Individuals responding with a positive DTH skin test to an *M. leprae* protein could then be tested for lepromin reactivity or lymphocyte blastogenic reactivity to *M. leprae* to give a more precise characterization of their ability to mount an immune response to *M. leprae*. Together, these tests could identify those individuals at highest risk for developing Hansen's disease and, thereby, focus subsequent patient monitoring and treatment on those individuals.

## SUMMARY

The cell wall-associated protein of *Mycobacterium gordonae* ($M_r$ = 65,000) was purified by affinity chromatography using a murine monoclonal antibody produced in response to the crossreactive 65 kD protein of *M. leprae*. The affinity-purified material was analyzed for purity by protein and carbohydrate analyses, SDS-PAGE, and immunoblotting. The final preparation contained a major protein band on SDS-PAGE analysis ($M_r$ = 65,000) with no detectable carbohydrates. The affinity fraction was prepared at 250 μg/ml (protein) in sterile saline and 0.1 ml injected intradermally into guinea pigs immunized 30 days earlier. Gross changes at 48 hr were consistent with the characteristics of a delayed hypersensitivity skin reaction measuring 2.5 mm, 3.4 mm, and 2.7 mm in animals which had been immunized with *M. leprae, M. gordonae,* or *M. bovis* (BCG), respectively. Histologically, all 65 kD protein skin-test sites showed marked edema and infiltration by numerous lymphocytes, macrophages, and scattered neutrophils. Animals injected with Freund's incomplete adjuvant showed a minimal or no reaction (1.8 mm) to the purified protein. These results further define the immunogenicity of the 65 kD protein of *M. gordonae* and by inference *M. leprae,* and demonstrate the ability of crossreactive epitopes of the 65 kD protein to sensitize lymphocytes involved in delayed-type hypersensitivity reaction to *M. leprae, M. gordonae,* and *M. bovis* (BCG).

## RESUMEN

Usando un anticuerpo monoclonal contra la proteína 65 kD del *Mycobacterium leprae* y cromatografía de afinidad, se aisló la proteína 65 kD asociada a la pared del *M. gordonae*. La pureza del material aislado por afinidad se estableció por análisis de proteínas y carbohidratos, por electroforesis en gel de poliacrilamida con dodecil sulfato de sodio, y por inmunotransferencia. La preparación final mostró una banda principal en la electroforesis en gel de poliacrilamida (P.M. =

65 kD) sin carbohidratos detectables. La fracción purificada se ajustó a 250 μg/ml (proteína) en solución salina estéril, y 0.1 ml de la solución se inyectó intradérmicamente en cobayos inmunizados 30 días antes. Los cambios gruesos a las 48 horas fueron consistentes con las caracteristicas de una reacción de hipersensibilidad tardía de dimensiones 2.5 mm, 3.4 mm y 2.7 mm en los animales que habían sido inmunizados con *M. leprae, M. gordonae,* o *M. bovis* (BCG), respectivamente. Histológicamente, todos los sitios de inoculación de la proteína 65 kD mostraron marcado edema e infiltración por numerosos linfocitos, macrófagos y algunos neutrófilos. Los animales inyectados sólo con adyuvante incompleto de Freund mostraron una reacción mínima (1.8 mm) o no reaccionaron contra la proteía purificada. Estos resultados definen en poco más la inmunogenicidad de la proteína 65 kD del *M. gordonae* y por inferencia, de la del *M. leprae,* y demuestran la capacidad de los epitopes de reacción cruzada de la proteína 65 kD para sensibilizar a los linfocitos involucrados en las reacciones de hipersensibilidad retardada contra *M. leprae, M. gordonae* y *M. bovis* (BCG).

## RÉSUMÉ

La protéine associée à la paroi cellulaire de *Mycobacterium gordonae* ($M_w$ = 65.000 daltons) a été purifié par chromatographie d'affinité, au moyen d'un anticorps monoclonal murin produit à la suite d'une sensibilisation par la protéine de *M. leprae* de 65 kD qui réagit de manière croisée. Le matériel purifié par cette méthode d'affinité a été analysé pour sa pureté par des analyses des protéines et des hydrates de carbone, par la méthode SDS-PAGE, et par la méthode dite "d'immunoblotting." La préparation finale contenait une bande principale protéinique lors de l'analyse par la méthode SDS-PAGE ($M_w$ = 65 kD); aucun hydrate de carbone ne pouvait être mis en évidence. La fraction d'affinité a été préparée à la concentration de 250 μg/ml (protéine) dans du serum physiologique stérile; cette fraction a été injectée à la dose de 0,1 ml, par voie intradermique, chez des cobayes immunisés 30 jours auparavant. Les changements macroscopiques décelés après 48 heures correspondaient aux caractéristiques d'une réaction cutanée d'hypersensibilité retardée; les réactions mesuraient respectivement 2,5 mm, 3,4 mm, et 2,7 mm chez les animaux qui avaient été respectivement immunisés au préalable par *M. leprae, M. gordonae,* ou *M. bovis* (BCG). Au point de vue histologique, tous les endroits de réaction cutanée à la protéine 65 kD montraient un oedème notable, ainsi qu'une infiltration par de nombreux lymphocytes et macrophages, et par des neutrophiles dispersés. Les animaux injectés avec l'adjuvant incomplet de Freund n'ont témoigné d'aucune réaction, ou d'une réaction minimale (1,8 mm) à la protéine purifiée. Ces résultats permettent de définir davantage le caractère immunogénique de la protéine 65 kD de *M. gordonae,* et d'en déduire dès lors celui de *M. leprae.* Ils démontrent la capacité des épitopes de réaction croisée situés sur la protéine de 65 kD, à sensibiliser les lymphocytes qui sont impliqués dans des réactions d'hypersensibilité de type retardé à *M. leprae,* à *M. gordonae,* et à *M. bovis* (BCG).

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