Studies with mAbs have confirmed the earlier observation (1) that Mycobacterium bovis (Bacillus Calmette-Guerin [BCG] strain), M. tuberculosis, and M. leprae share a number of immunoreactive molecules. Murine mAbs raised against M. leprae react with carbohydrate and protein antigens common to both mycobacteria, as well as apparently M. leprae-specific antigens (2–6). Recently (7), the DNA encoding five peptide antigens of M. leprae has been isolated using a panel of mAbs in the λgt11 expression system, and two of these proteins, with Mr 65,000 and 28,000, were shown to possess epitopes common to M. leprae and M. bovis. Recently (5), we described another mAb, L7, reacting with a 70 kD protein in crude M. leprae sonicate that is more abundant in M. bovis and M. tuberculosis preparations.

The role of the mAb-defined determinants in the human B cell response has been examined (8) by using leprosy sera in mAb inhibition assays. For example, we showed (5) that the inhibitory titer was higher for antibodies directed against cell wall–associated carbohydrate molecules than for those recognizing proteins of Mr 18,000 and 70,000. However, protection against mycobacteria is mediated by T cell responses (9), and purified homogeneous antigens are therefore required for cellular studies to elucidate the molecular basis of protective immunity. These antigens may be derived through recombinant DNA technology by purification of peptides from a suitable expression vector such as Escherichia coli (10), or by the preparation of synthetic peptides predicted on the basis of the DNA sequence of individual genes. The alternative approach adopted here is to use mAb solid-phase affinity chromatography to separate the native antigen from a soluble preparation of the mycobacteria. The antigen selected was the p70 crossreactive protein recognized by L7. In view of the limited supply of armadillo-derived M. leprae, the cultivatable mycobacteria M. bovis served as the antigen source. After purification on an L7 affinity column, the p70 antigen was found to react with sera from lepromatous leprosy patients, to stimulate peripheral
blood mononuclear cells (PBMC) from mantoux-positive individuals with release of IFN-γ and to elicit a cutaneous delayed-type hypersensitivity (DTH) reaction.

Materials and Methods

Antigens. M. bovis (BCG) was obtained as freeze-dried whole bacilli from the Commonwealth Serum Laboratories (Melbourne, Australia) and M. leprae (preparation CD45) was kindly provided by Dr. R. J. W. Rees as part of the immunology of Leprosy (IMMLEP) program. Sonicates of both organisms were prepared as described (5) previously. PPD was obtained from the Statens Seruminstitut (Copenhagen, Denmark).

Antibodies. L7, an IgG1 mAb, was derived from a fusion of P3-NS1-Ag4-1 myeloma cells with spleen cells from BALB/c/J mice immunized with M. leprae sonicate (5). The antibody was purified from ascites fluid by ammonium sulfate precipitation and protein A-Sepharose chromatography (11). The human serum pools used were from 10 Nepali patients with untreated lepromatous and borderline lepromatous leprosy, and from 10 untreated smear-positive patients with pulmonary tuberculosis. Control sera were obtained from a mantoux-negative healthy Caucasian resident in Sydney.

mAb Affinity Chromatography. L7 was coupled to cyanogen bromide-activated Sepharose 4 B (Pharmacia Fine Chemicals, Uppsala, Sweden) at a ratio of 5 mg of antibody to 1 ml gel in 0.25 M sodium bicarbonate buffer (pH 9.0) containing 0.5 M sodium chloride for 2 h at room temperature. The gel was then reacted with 1 M ethanolamine for 2 h at room temperature before washing alternately with 0.1 M sodium acetate (pH 4.0) containing sodium chloride (0.5 M) and coupling buffer for four cycles. The washed gel was then stored in 0.15 M PBS, pH 7.2, containing 0.1% sodium azide at 4°C. Normal mouse (BALB/c/J) Ig purified by protein A-Sepharose chromatography was coupled to Sepharose 4 B in the same way.

M. bovis sonicate (23.5 ml, 5 mg/ml) was first passed through a normal mouse Ig-Sepharose 4 B column and recirculated for 1 h at a flow rate of 2 ml/min. Subsequently, unbound material was recirculated through a L7 Sepharose 4 B column for 2 h at the same flow rate. This column was then washed extensively with PBS containing 0.65 M sodium chloride until a stable baseline was obtained. The bound fraction was eluted with 0.05 M diethylamine, pH 11.5, (BDH Chemicals, Poole, England), pooled and dialyzed against 0.015 M PBS before immunoprecipitation. The lyophilized material was reconstituted in one-tenth of its original volume of sterile water, and the protein concentration was determined with a spectrophotometer (12). The solution was filtered through a 0.22 μm filter (Millipore, Bedford, MA) before in vitro or in vivo use.

SDS-PAGE and Immunoblotting. The eluted and unbound material, and the original M. bovis sonicate (10 μg protein per track) were subjected to 6–20% gradient SDS-PAGE (13). The protein bands were stained with Coomassie blue (R250; Biorad Laboratories, Richmond, CA) or transferred to nitrocellulose paper (Biorad Laboratories) for immunoblotting with L7 as described previously (5). The second antibody used was 125I-labelled sheep anti-mouse Ig, and the nitrocellulose paper strips were developed by autoradiographic exposure to Kodak XRP-I film (Kodak, Melbourne, Australia) at −70°C. The 125I-labelled p70 antigen was also analyzed by one- and two-dimensional PAGE before and after immunoprecipitation with test sera (see below). Two-dimensional analysis involved separation in the first dimension by IEF in 7.5% polyacrylamide containing 7.6% pharmalyte, pH range 4–6.5 (Pharmacia Fine Chemicals), 2% NP-40, and 4.5 M urea. This was followed by electrophoresis in the second dimension using 6–20% SDS-PAGE (14). The gels were stained with Coomassie blue and dried onto filter paper before development by autoradiography for 24–48 h. The molecular weight markers ranged from 93,000 to 14,400 (Pharmacia Fine Chemicals).

Immunoprecipitation. The eluate from the L7 column containing the p70 antigen (500 μg/ml, 100 μl) and M. lepra or M. bovis (BCG) sonicate (1 mg/ml, 100 μl) were radiolabelled with 125I (Amersham Corp., Buckinghamshire, United Kingdom) by the chloramine T method (15). The radiolabelled antigen was immunoprecipitated with the test sera as described by Brown and coworkers (16). Briefly, 4 × 108 cpm of 125I-p70 or
<125*I-labeled M. bovis (BCG) sonicate in 200 µl 0.2% NP-40/PBS were incubated with 10 µl serum for 2 h on ice in a microfuge tube. Staphylococcus aureus, Cowan strain A (Staph. A; heat-killed, formalin fixed; 10% in PBS; Commonwealth Serum Laboratories, Melbourne, Australia) was added in 500 µl with agitation before incubation on ice for 30 min. The antigen-antibody Staph. A complex was washed vigorously three times with NP-40/PBS. The antigen was then released by incubation with electrophoresis sample buffer containing 4% SDS, 4% 2-ME, 20% sucrose in 0.05 M Tris-NCI, pH 6.8 for SDS-PAGE, or with 2% SDS, 2% NP-40, 9.5 M urea for IEF. After centrifugation, a 5 µl sample of the supernatant was counted before electrophoresis.

ELISA with p70 Antigen. The p70 fraction was diluted in PBS to concentrations of 100–0.01 µg/ml, and the wells of a polystyrene microtiter tray (Linbro; Flow Laboratories, Edinburgh, United Kingdom) were coated with 50 µl of each dilution by incubation for 2 h at 37°C. The wells were then blocked with 3% BSA (Miles Laboratories, Naperville, IL) in PBS/azide for 1 h. Aliquots of pooled leprosy sera, tuberculosis sera, and control sera (50 µl) were added to triplicate wells of each antigen concentration at a dilution of 1:300 in 0.1% BSA/PBS/Tween 20 for 1 h. After extensive washing, the wells were pulsed with goat anti-human IgG conjugated with alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) diluted 1:1,000 in 0.1% BSA/PBS/Tween 20 and incubation continued for a further hour. Color was developed by the addition of the enzyme substrate, nitrophenyl phosphate (Sigma Chemical Co.), and the ODs were read at 30 and 60 min in a Titertek Multiscan (Flow Laboratories) at 405 nm.

Antigen Inhibition Assay. Doubling dilutions of the purified p70 and the original M. bovis sonicate were added to BSA-blocked round-bottom wells in a microtiter tray together with an equal volume of fresh L7 hybridoma culture supernatant for incubation at 37°C for 1 h. 50 µl of each dilution was transferred in triplicate to microtiter tray wells which were coated with M. bovis sonicate (50 µg/ml) and then with 3% BSA. This was followed by alkaline phosphatase–conjugated sheep anti–mouse Ig (New England Nuclear, Boston, MA) and enzyme substrate. The mean OD of wells incubated with BSA alone was subtracted from that of each test well. Since maximum binding occurred with L7 diluted 1:2, the percentage inhibition of maximum binding achieved at each antigen concentration could be calculated.

Human T Cell Reactivity. PBMC were separated from heparinized venous blood of subjects by centrifugation over Ficoll/Hypaque (17). After washing with RPMI 1640 (Flow Laboratories) containing 10 mM sodium bicarbonate and 25 mM Hepes, the cells were adjusted to a concentration of 10⁶ cells/ml with RPMI containing 20% heat-inactivated (A-) human serum, penicillin (50 mg/liter) and streptomycin (100 mg/liter). 200 µl aliquots containing 2 × 10⁵ cells were added to the wells of flat-bottom microtiter trays (Linbro; Flow Laboratories) followed by various concentrations of antigens in 25 µl of RPMI. In the case of p70 and M. bovis, the final concentrations were 10, 3, 1.0, 0.3, and 0.1 µg/ml, whereas for PPD they were 10, 1.0, and 0.1 µg/ml. After 5 d incubation at 37°C in 5% CO₂, each well was pulsed with 1.0 µCi of [³H]thymidine (Amersham Corp.), and the cells were harvested 16 h later. [³H]thymidine incorporation was measured in dpm by liquid scintillation spectroscopy (1218 Rackbeta; LKB Instruments, Gaithersburg, MD). Before addition of [³H]thymidine, 100 µl of supernatant was removed from the triplicate wells and replaced with 100 µl of fresh medium. The 100 µl samples were then pooled and stored at −20°C for subsequent measurement of IFN-γ activity (18).

IFN-γ activity was measured by a cytopathic effect–inhibition assay using the method of Ho and Enders (18). HEp-2 cells were grown to confluence in microtiter trays. IFN standards and samples were diluted and incubated with the cells for 24 h at 37°C. The supernatants were then removed and replaced with 4 × 10⁴ PFU/well of Semliki Forest virus. After a further 48 h incubation at 37°C, the cells were fixed and stained with neutral red. Endpoint dilutions were read at 50% protection of cytopathic effect inhibition. The human reference IFN-γ was supplied by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (Bethesda, MD).

For DTH skin testing, the p70 antigen was adjusted to a final concentration of 1 µg/ml in sterile PBS. 100 µl was injected intradermally on the volar aspect of the forearm, and
the horizontal and vertical diameters were measured in millimeters 48 and 72 h later. The PBS diluent was also injected intradermally for comparison.

Subjects. Ethical approval for the use of the purified p70 antigen was obtained from the University of Sydney Human Studies Ethics Committee. The subjects consisted of healthy volunteers who were skin tested with tuberculin PPD (100 U/ml; Commonwealth Serum Laboratories). 12 had received BCG vaccinations and were PPD+, 7 were naturally PPD+, and 8 were PPD- without prior vaccination or known contact with tuberculosis.

Amino Acid Sequencing. The p70 antigen (~25 µg) was analyzed on a Model 890A gas-phase sequencer (Applied Biosystems, Inc., Foster City, CA). It was first dissolved in 98% formic acid (20 µl) and loaded into a glass fiber disc that had been pretreated with trifluoroacetic acid. The phenylthiohydantoins (PTHs) were identified by HPLC (19).

Results

Separation of p70 Antigen by Solid-phase Affinity Chromatography. From 117.5 mg of M. bovis sonicate applied to the L7-Sepharose 4 B column, 1.6 mg (1.3%) were eluted with alkaline (pH 11.5) buffer. Staining of SDS-PAGE-fractionated samples (10 µg) revealed a marked increase in the density and size of the 70 kD band in the pH 11.5 eluate compared to the faint band seen in tracks of the original sonicate, while the unbound material (PBS fraction) was depleted of this band (Fig. 1A). There was a faint second band of M, 68,000 beneath the major band, and this pattern resembled the doublet of L7-reactive bands obtained previously (5) on immunoblots of crude M. leprae and M. bovis sonicates. In addition, when 20 µg of pH 11.5 eluate was applied, faint bands were present with M, 60,000 and 32,000. The increased concentration of the L7 reactive protein in the pH 11.5 eluate was confirmed by immunoblotting, and faint reactivity was present in the region of M, 60,000 and 32,000 (Fig. 1B), consistent with these being fragments of the p70 antigen. Once again the PBS fraction contained no demonstrable p70 antigen. The reactions with the original sonicate were relatively weak, which was consistent with the results obtained in earlier experiments (5) comparing various anti-M. leprae mAbs. Examination of the pH 11.5 eluate from the normal mouse Ig column revealed faint protein bands of
M, 55,000 and 27,000, but no immunoreactive p70 on immunoblotting with L7 (data not shown).

Reactivity of p70 with the L7 mAb. Enrichment of p70 in the column eluate was confirmed in the antigen-inhibition ELISA. Using crude sonicates of M. leprae or M. bovis, high protein concentrations (500–1,000 µg/ml) were required to inhibit the binding of L7 to antigen-coated wells. With the pH 11.5 fraction, the inhibition curve was shifted to the right (Fig. 2) and the concentration causing 50% inhibition of binding of L7 to M. bovis was 20 µg/ml, compared to 750 µg/ml for the whole sonicate.

Reactivity of p70 with Leprosy and Tuberculosis Sera. Incubation of ¹²⁵I–M. leprae sonicate with the pool of leprosy sera had indicated that the M, 70,000 band was one of 13 bands immunoprecipitated by human immune sera. L7 precipitated a similar band from M. leprae and M. bovis sonicate in a solid-phase immunoprecipitation assay (data not shown). Therefore, we examined the reactivity of p70 with antisera from patients with leprosy and tuberculosis. In the direct ELISA, human leprosy sera reacted with the p70 fraction diluted to 0.01 µg/ml, whereas 1 µg/ml was required for significant binding to tuberculosis sera. At each dilution, the leprosy serum pool was more reactive than the tuberculosis sera pool with the p70 antigen (Fig. 3).

The p70-containing fraction was radiolabelled with ¹²⁵I. Autoradiography after SDS-PAGE revealed a dense band at M, 70,000 with faint bands at 67,000 and 35,000. Two-dimensional electrophoresis showed that the radiolabelled p70 had
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FIGURE 3. Direct ELISA using p70 purified from L7 column. The serum pools were incubated with p70 at different concentrations and the ELISA developed. The serum pools were derived from patients with lepromatous leprosy (●), tuberculosis sera (○), or normal mantoux-negative controls (△).

FIGURE 4. Two-dimensional electrophoresis with separation in the horizontal dimension by IEF, and in the vertical dimension by mol wt with SDS-PAGE. IEF markers are, from the cathode end, pH 5.85, 5.20, 4.55, and 4.15. M, markers (× 10^-5) are on the left side. A. 10^6 cpm of ^125I-labelled pH 11.5 eluate from L7 column. B. The same antigenic material immunoprecipitated by lepromatous leprosy sera (see text).

a pI of 5.35 (Fig. 4A). After incubation with the leprosy serum pool, 17,778 cpm per 5 μl of the ^125I-p70 (5.3%) were subsequently eluted from the Staph. A complex and compared to 6,019 cpm per 5 μl (1.8%) and 5,484 cpm per 5 μl (1.6%) after incubation with tuberculosis and control sera, respectively. Two-dimensional electrophoresis of the eluted material confirmed that p70 had been
precipitated by the leprosy sera (Fig. 4B). In addition, a faint $M_r$ 35,000 band was precipitated by the immune sera.

Comparison of $^{125}$I-p70 with $^{125}$I-M. bovis sonicate examined under similar conditions showed the enrichment of p70 by affinity chromatography (Fig. 5). >50 bands were visible in M. bovis (BCG) sonicate, and 20 of these were precipitated by leprosy sera. $^{125}$I-p70 migrated to the same position as one of these bands (Fig. 5A). A similar protein was identified by immunoprecipitation of $^{125}$I-M. leprae sonicate with either L7 or leprosy sera (data not shown).

**In Vitro T Cell Reactivity.** The presence of T cell–reactive epitopes on the purified p70 antigen was tested in vitro by proliferation and IFN-$\gamma$ release, and in vivo by DTH. Initially, the cellular reactivity of the purified p70 antigen was compared with the original M. bovis sonicate in a proliferative assay over a dose range of 0.1–10 $\mu$g/ml (Fig. 6). Among the eight controls with no exposure to tuberculosis and no skin test reactivity to PPD, only one had a mild proliferative response to p70 and M. bovis. By contrast, marked proliferation to the p70 antigen and M. bovis sonicate at concentration 10–0.3 $\mu$g/ml was observed in both BCG-vaccinated subjects and those with natural immunity to M. tuberculosis, the highest response occurring in the latter group. In a few individuals, the response to p70 and M. bovis at the same concentration was comparable, but in the majority of cases the p70 response was 50–70% of that to whole sonicate (Table I).

The release of IFN-$\gamma$ into the culture supernatant during the proliferative response to p70 and M. bovis was measured in some subjects (Table II). The lymphokine was only detected in supernatants derived from cultures in which the cells had responded to p70 or M. bovis. However, the higher concentration of 10 $\mu$g/ml was required for detectable IFN-$\gamma$ to be present in cultures from all the skin test–positive individuals (Table II).

**DTH Reactivity of p70.** The in vivo response to p70 was examined in the same three groups of subjects. Initial observations with p70 at concentrations of 1.0–
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FIGURE 6. Proliferative responses of PBM to p70 antigen. Individual points represent the mean ± SEM of the Δdpm at that antigen concentration for the members of each group. Naturally mantoux-positive (▲); BCG-vaccinated (■); Mantoux-negative controls (●).

TABLE I

T Cell Responses to M. Bovis Sonicate and Purified p70 Antigens

| Group Number | DTH skin test (mean diameter ± SEM; mm) for M. bovis p70 (1 µg/ml inoculum) | Lymphocyte proliferation ([3H]thymidine incorporation; Δ dpm mean ± SEM) for inoculum of: |
|--------------|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| 1            | 8 | 0 | 1,952 ± 536 | 1,899 ± 575 |
| 2            | 12 | 8.8 ± 1.4 | 24,545 ± 8,519* | 40,422 ± 10,806* |
| 3            | 7 | 12.2 ± 2.6 | 42,187 ± 12,812* | 84,714 ± 29,398* |

Groups were: 1, naive recipients; 2, BCG-vaccinated recipients; 3, recipients with natural reactivity to PPD.
* Significantly different from naive recipients at p < 0.01.
† Significantly different from naive recipients at p < 0.005.
**Table 11**

*In Vitro IFN-γ Production to p70 and *M. bovis*

| Group | Number | IFN-γ (IU/ml; mean ± SEM) for inocula of: |
|-------|--------|------------------------------------------|
|       |        | *M. bovis* p70                           | *M. bovis*                        |
|       |        | 10 µg/ml | 1 µg/ml | 10 µg/ml | 1 µg/ml |
| 1     | 4      | 0        | 0       | 0        | 0       |
| 2     | 7      | 983 ± 309 (7)* | 103 ± 90 (2) | 297 ± 65 (7) | 46 ± 45 (1) |
| 3     | 6      | 1,066 ± 135 (6) | 386 ± 205 (4) | 695 ± 212 (6) | 533 ± 257 (4) |

Measured by CPE-inhibition assay. Groups as in Table I.

*Number of individuals responding is given in parentheses.

0.01 µg/ml indicated that, although some strongly reactive subjects responded to p70 at concentrations of 0.01 µg/ml, other PPD+ individuals only did so at 1 µg/ml. Therefore, 100 µl of p70, 1 µg/ml, was selected as the test dose for intradermal injection into the 27 subjects. There were no responses to the PBS diluent. Mantoux-negative individuals had no skin test reactivity to p70, including the subject with a mild proliferative response to p70 (Table I). A number of the responsive subjects (4 of 19) displayed an initial flare reaction, which subsided after 12 h, but the cutaneous oedema reaction peaked at 48–72 h and then subsided. The results at 48 h indicated that BCG-vaccinated subjects reacted to a lesser extent than those with natural reactivity to PPD. When the relationship between skin test reactivity and proliferative response at 1 µg/ml was examined, a moderately significant correlation was observed between the two responses (Spearman rank coefficient, 0.708).

**Partial Amino Acid Sequence.** The N-terminal sequence of the p70 antigen was analyzed by a gas-phase sequenator; the first 15 amino acids are: Ala-Arg-Ala-Val-Gly-Ile-Asp-Leu-Gly-Thr-Asp-Asn-Ser-Val-Val. The chromatograms showed a persistent background of PTH-amino acids (particularly PTH-aspartic acid), but the major peak (except for cycle 2) was always at least double the height of background peaks. While PTH-arginine was not the largest peak in cycle 2, relative to cycle 1 it increased the most, and because this derivative is less easily extracted from the sequencer than others, residue 2 was assigned as arginine.

**Discussion**

Mycobacteria such as *M. leprae*, *M. bovis*, and *M. tuberculosis* present multiple antigens to the infected host (20). These are derived from the complex cell wall surrounding the bacterium with its matrix of polysaccharides and peptides linked to a peptidoglycan backbone, or are released from the cytoplasm (21). Recent studies (6, 22) with mAbs raised against these three mycobacteria have led to the identification of a number of glycolipid, carbohydrate, and protein antigens. Among these mAbs was L7, which recognized an antigen of *M. 70,000* (p70), its distribution being restricted to *M. leprae*, *M. bovis*, *M. tuberculosis*, and *M. scrofulaceum* within the panel of 14 mycobacteria tested (5). The antigen was a protein, as indicated by its sensitivity to subtilisin and trypsin and its resistance to periodate oxidation, but it was not associated with the mycobacterial cell wall. p70 is distinct from the 60–65,000 complex identified by Gillis and coworkers.
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(23) and ourselves as a cell wall–associated peptide. The excellent separation of p70 achieved with L7 from either *M. bovis* or *M. leprae* sonicates in a solid-phase immunoprecipitation assay suggested that it would be a suitable antibody for affinity chromatography. This was substantiated when the material eluted from the L7-Sepharose column was shown to consist predominantly of the p70 antigen (Fig. 1). Two-dimensional electrophoresis of whole BCG sonicate and p70 showed the relative homogeneity of the purified antigen. The minor *M. 32,000 and 60,000 bands were reactive with L7 and were thought to reflect the effect of proteolytic enzymes on p70. Treatment of crude sonicate with trypsin before immunoblotting with L7 showed that p70 could be cleaved into L7-reactive fragments (data not shown). The antigenic activity of the pH 11.5 fraction from the L7-Sepharose column was therefore considered to reside in the dominant p70 protein.

The enhanced reactivity of the pH 11.5 fraction in the antigen-inhibition assay (Fig. 2) confirmed the concentration of p70 observed in immunoblots. The antigen was shown to react directly with leprosy sera, which in conjunction with the results from the monoclonal inhibition studies indicated that the L7 determinant was recognized by human B cells (Fig. 3). Interestingly, p70 was readily detected by immunoprecipitation with human sera, whereas it was not well seen in immunoblots (5, 25, 26). These findings illustrate the different sensitivity of the two techniques. Determinants on other proteins of *M. leprae*, including the *M. 18,000, 36,000, and 65,000 antigens have been shown previously (5, 8) to be human B cell immunogens in mAb inhibition assays. Recently (27) this has been validated in the case of the 65,000 antigen with direct binding studies using the homologous protein purified from *M. gordonae*.

The protective response to mycobacteria is mediated by activated T cells and their products rather than antibodies (9). Evidence of a role for the p70 antigen in cellular immune responses was obtained from both in vitro and in vivo studies. When PBM from manchoux-positive donors were cultured in the presence of p70 or *M. bovis* sonicate, a dose-dependent proliferative response occurred to both antigen preparations, with release of IFN-γ (Fig. 6, and Tables I and II). The degree of proliferation to p70 was 50–100% of that to the original sonicate, indicating that p70 is likely to be a significant T cell immunogen. Although it will be necessary to carry out limiting-dilution analysis to prove this conclusively, the DTH reactivity of p70 strongly supports its importance in T cell responses to *M. bovis*, and probably to *M. tuberculosis* and *M. leprae* as well. Evidence in favor of this comes from the observation that human T cell clones with cross-reactivity for *M. bovis* and *M. leprae* underwent substantial proliferation in the presence of purified p70 (data not shown).

The cellular activity of p70 from *M. bovis* in man suggests that it has the potential to stimulate protection against infection with *M. bovis, M. tuberculosis*, and possibly *M. leprae*. It will be possible to test this in the case of *M. tuberculosis* by using a murine model of infection in which protection is assayed in mice immunized with p70 or *M. bovis* sonicate. With *M. leprae* infection, p70 purified from *M. bovis* may mimic the protection achieved with whole *M. bovis* in the mouse footpad assay (28). However, it will be more relevant to test p70 derived from *M. leprae* itself in this system, when it becomes available, possibly through
recombinant DNA technology (vide infra). In the interim, p70 purified from *M. bovis* is being tested for its ability to elicit DTH in subjects with tuberculosis and in leprosy patients across the clinical spectrum.

Other well-characterized mycobacterial antigens have been recently shown to express T cell reactive epitopes. For example, others (29, 30) have isolated human T cell clones responding to an antigenic fraction purified from *M. tuberculosis* with mAb TB 68. This antigen was identified as a molecule of *M.* 120,000, which after SDS denaturation resolved into four bands, each of which was distinct from the p70 antigen described here. Using a different mAb directed against *M. bovis* (BCG), Minden and coworkers (31) purified a 10,000 *M.* protein and showed that it had DTH reactivity in sensitised guinea pigs. Recently, Mustafa and coworkers (32) examined the T cell reactivity of five *M. leprae* peptides expressed in *E. coli* with the λgt11 system. They sought to circumvent the need to purify individual antigens from the expression vector by using *E. coli* lysates, which they tested against a panel of T cell clones derived from subjects vaccinated with *M. leprae*. *M. leprae*-specific T cell clones were stimulated only by *E. coli* lysates containing the epitope recognized by mAb (L7.15), shown previously (5) to identify an *M. leprae*-specific determinant on an *M.* 18,000 protein. This finding did not exclude the possibility that the other four peptide antigens of *M.* 65,000, 36,000, 28,000, and 12,000 were also T cell immunogens, since only a relatively small number of T cell clones were tested. In fact, using proteins purified with mAb affinity chromatography or HPLC, both the *M.* 36,000 and 12,000 antigens were shown (33) to stimulate human T cell clones obtained from a patient with tuberculoid leprosy. Although the 36,000 protein had been defined with an *M. leprae*-specific mAb, only one of the four responsive T cell clones was *M. leprae*-specific, indicating that common mycobacterial determinants were also present on the 36,000 protein.

A recent IMMTUB (Immunology of Tuberculosis) workshop (34) compared the reactivity of anti-*M. tuberculosis* mAbs, and sought to identify DNA clones of *M. tuberculosis* expressing mAb-defined epitopes using the same bacteriophage vector, λgt11. Among the 31 mAbs tested, one (51A) reacted with a band of 71,000 *M.* and showed a limited pattern of crossreactivity. 51A identified a 48,000 band in *M. leprae* immunoblots, suggesting that it recognized a different epitope from that defined by L7 (O. Closs, personal communication). DNA clones of *M. tuberculosis* encoding the epitope defined by 51A in *M. tuberculosis* sonicate immunoblots have been isolated (34). It remains to be established whether L7 recognizes the protein expressed by these clones or whether they crosshybridize with the two DNA clones recently isolated from the *M. leprae* genomic library with L7 by J. D. Watson (Auckland Medical School, Auckland, New Zealand; personal communication). If the proteins are related, purification of the *E. coli*-expressed 71,000 *M. tuberculosis* protein in whole or part will allow comparison between the cellular reactivity of the native p70 protein and the *E. coli* expression product. Ultimately, such studies will elucidate the role of the p70 antigen in stimulating protective responses to *M. leprae*, *M. tuberculosis*, or both bacteria in infected individuals, and will determine whether it might be a suitable candidate for inclusion in a polyspecific vaccine.
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Summary

The protein antigens from Mycobacterium bovis (BCG), M. tuberculosis, and M. leprae share a number of common determinants. We have used a murine mAb (L7) recognizing such a determinant on a protein of Mr 70,000 to purify this antigen from M. bovis sonicate by affinity chromatography. Enrichment of the protein in column eluates was confirmed by immunoblotting and in antigen inhibition assays. After radiolabelling with $^{125}$I, the protein could be immuno-precipitated with human lepromatous leprosy sera. Stimulation of peripheral blood mononuclear cells from BCG-vaccinated and naturally mantoux-positive individuals induced proliferation and IFN-γ secretion, while intradermal injection of purified antigen into the same subjects resulted in a delayed-type hypersensitivity reaction. Thus, the 70,000 molecule carried epitopes capable of reacting with B cells, and eliciting a potentially protective T cell response. The first 15 N-terminal residues were sequenced using a gas-phase sequenator.

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