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# Analysis of B-Cell Epitopes in the Variable C-Terminal Region of the *Mycobacterium leprae* 70-Kilodalton Heat Shock Protein

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**The C-terminal region of the *Mycobacterium leprae* 70-kDa heat-shock protein is the major target for the humoral immune response to this protein and contains *M. leprae*-specific sequences. To examine B-cell responses to this region more closely, we constructed and expressed a recombinant fragment of the *M. leprae* P70 gene that encodes the C-terminal 142 residues (C-142) and synthesized a series of 10 overlapping peptides to encompass this region. The affinities of three monoclonal antibodies (MAbs) reactive with this region of P70 were measured, and the binding site of the highest-affinity MAb was determined to lie between residues 498 and 515. This reactivity was confirmed by a fluid-phase inhibition enzyme-linked immunosorbent assay. By contrast, sera from leprosy patients which were strongly reactive with the C-142 fragment failed to bind directly to the conjugated or unconjugated peptides. To determine whether the *M. leprae*-specific C-terminal 70 residues could stimulate B-cell responses, the reactivity of hyperimmune anti-*M. leprae* P70 antisera with the peptides was examined. Rabbit polyclonal anti-*M. leprae* P70 antisera recognized epitopes between residues 498 and 515 and in the *M. leprae*-specific region between residues 567 and 591. The latter, in turn, when coupled to ovalbumin, was able to generate a strong anti-P70 response specific for mycobacterial, but not human, HSP70. Three strains of mice immunized with either C-142 or P70 recognized epitopes in the region between residues 487 and 532, but the response varied with the strain and immunogen. These data demonstrate that two regions in the C-terminal portion of *M. leprae* P70 contain linear B-cell epitopes recognized by MAbs or hyperimmune serum. Sera from leprosy patients, however, react predominantly with conformational determinants in the immunodominant C-terminal part of the protein.**

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. The lack of a universally effective vaccine against *M. leprae* infection and the paucity of reliable tests for the diagnosis of subclinical infection have led to the detailed study of the ability of *M. leprae* antigens to evoke host immune responses. This process was facilitated by the development of a recombinant λgt11 library of *M. leprae* DNA (7) and the subsequent isolation of genes that encode proteins recognized by monoclonal antibodies (MAbs) (30). The initial panel of 70-, 65-, 36-, 28-, and 18-kDa (3, 12, 19, 24, 25) proteins has been expanded to include over 12 individual proteins (29). The definition of B- and T-cell epitopes on immunodominant proteins may ultimately permit the development of specific diagnostic tests and novel protective vaccines.

The 70-kDa protein of *M. leprae* (P70) was initially identified with murine MAb L7 (6), which recognized a similar antigen in *M. bovis* bacillus Calmette-Guérin (BCG) and *M. tuberculosis*. Despite a high degree of structural homology (10, 18, 20, 28) with the 70-kDa family of prokaryotic and eukaryotic heat shock proteins (HSP70), the BCG and *M. leprae* 70-kDa proteins induced strong T- and B-cell responses in humans (1, 5, 28). The homology between mycobacterial and mammalian HSP70 proteins declines at the C-terminal end of the molecule, and there is complete divergence of the *M. leprae* and *M. tuberculosis* sequences in the C-terminal 70 residues (18). The C-terminal region of the molecule is the major target for humoral immune responses (8) and thus may contain species-specific B-cell epitopes. To investigate this possibility, a recombinant fragment of the *M. leprae* P70 gene that encodes the C-terminal 142 residues (C-142) was expressed and purified and 10 overlapping synthetic peptides were prepared to span this fragment. The reactivity of murine anti-P70 MAbs and human, rabbit, and murine polyclonal sera with this region of the molecule was then determined.

## MATERIALS AND METHODS

**Antigens and peptides.** Plasmid pGEX-70 contains the gene for the *M. leprae* 70-kDa protein linked to the DNA that encodes the C-terminal portion of glutathione-*S*-transferase from *Schistosoma japonicum* (23). Plasmid pGT-142, which encodes the C-142 fragment of *M. leprae* P70, was derived from clone JKL2 (12), which contains the 3′ half of the P70 gene. A 426-bp fragment was generated by polymerase chain reaction with a proximal *Bam*HI site and an *Sma*I site distal to the stop codon and the fragment cloned into pGEX-2T (23) to produce plasmid pGT-142. pGEX-70 and pGT-142 were expressed in *Escherichia coli* MC1061, and recombinant P70 and the C-142 fragment of P70 were produced as glutathione-*S*-transferase fusion proteins following induction with 0.1 mM isopropyl-β-D-thiogalactopyranoside (Sigma, St. Louis, Mo.) for 3 h at 37°C. The fusion proteins were purified by glutathione agarose affinity chromatography and cleaved by thrombin to yield soluble proteins as previously described (18). The purity and immunogenicity of P70 and C-142 were confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis and immunoblotting (8). The *M. bovis* 70-kDa protein was prepared by MAb affinity chromatography (5). Human P70 was also expressed and purified in the pGEX-2T vector by using a plasmid that

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TABLE 1. Sequences of overlapping peptides P1 to P10, which encompass the C-terminal 144 residues of *M. leprae* P70

| Peptide | Residues | Sequence[a] |
|---|---|---|
| P1 | 597–621 | SAPGGGSNSTDDVLTRRWSTTNGSPK |
| P2 | 582–606 | IYEATQAASKVGGEASAPGGSNSTD |
| P3 | 567–591 | AMEKLGQDSQALGQAIYEATQAASK |
| P4 | 552–576 | AKTALGGTDISAIKSAMEKLGQDSQ |
| P5 | 537–561 | VPEDTLNKVEAAVAEAKTALGGTDI |
| P6 | 522–546 | EKFVKEQRETENGSRVPEDTLNKVE |
| P7 | 506–532 | EEADVRNQAETLVYQTEKFVKEQRETE |
| P8 | 498–515 | AEEDRKRREEADVRNQAE |
| P9 | 487–507 | IDRMVKDAEAHAEEDRKRREE |
| P10 | 478–496 | EGSGLSKEEIDRMVKDAEA |

[a] Residues are listed in the single-letter code starting from the C-terminal end. The complete divergence between *M. leprae* and *M. tuberculosis* P70 commences at residue 551.

encodes inducible HSP70 obtained from the American Tissue Culture Collection (15).

**Peptides.** The overlapping peptides (Table 1) were synthesized by standard FMOC (9-fluorenylmethyloxycarbonyl) procedures by using a Milligen 9020 synthesizer. After release and deprotection, they were washed exhaustively with cold ether and lyophilized. All peptides were analyzed by high-pressure liquid chromatography with a C4 column and elution with a linear gradient of 0 to 100% acetonitrile. When appropriate, their structures were confirmed by amino acid analysis and sequencing. The peptides were conjugated to bovine serum albumin (BSA; Sigma) by incubation of BSA and peptide, both at 1 mg/ml in phosphate-buffered saline (PBS) with an equal volume of 2% glutaraldehyde (Sigma) for 60 min at 4°C. The reaction was then quenched by addition of $NaBH_4$ to 10 mg/ml for 60 min at 4°C, followed by overnight dialysis against 0.15 M PBS (pH 7.2)–0.05% sodium azide (PBS-azide).

**MAbs and sera.** MAb L7 (immunoglobulin G1 [IgG1]) was raised against *M. leprae* sonicate (6), and MAbs B7 (IgG1) and B8 (IgG2a) were prepared following immunization of BALB/c mice with the affinity-purified *M. bovis* BCG 70-kDa protein (8). Gamma globulin fractions of ascites were biotinylated with NHS-LC-biotin (Pierce, Rockford, Ill.). Groups of three C57BL/6, BALB/C, and CBA mice were immunized by three intraperitoneal injections of 20 μg of P70 or C-142 at weekly intervals and bled 1 week after the final injection. For the first two injections, the antigen was emulsified in incomplete Freund's adjuvant (Commonwealth Serum Laboratories). Two rabbits were immunized with three injections of 200 μg of P70 in incomplete Freund's adjuvant at 2-week intervals, and sera were collected at 6 weeks. Similarly, two other rabbits were given three injections of 1 mg of an ovalbumin-peptide 3 conjugate to induce anti-P3 antibodies. Human leprosy sera were from untreated lepromatous leprosy patients classified in accordance with the Ridley-Jopling classification (21).

**Peptide enzyme-linked immunosorbent assays (ELISAs).** Peptides, conjugated peptides, P70, and C-142, at concentrations of 0.1 to 50 μg/ml in PBS-azide, were immobilized on activated polyvinyl chloride plates (Flow, Sydney, Australia) by incubation at 37°C for 2 h and then overnight at 4°C before being washed twice with PBS. Nonspecific binding was blocked by incubation with 1% BSA–PBS for MAb assays or 1% skim milk powder containing 10% fetal calf serum for hyperimmune sera. Undiluted supernatants were incubated in duplicate with antigen-coated wells for 1 h at 37°C and then overnight at 4°C. Hyperimmune sera were incubated at dilutions of 1:100 or 1:200 in 0.1% BSA–PBS for the same period. Bound immunoglobulin was detected by further incubation with 1:1,000-diluted biotinylated species-specific antibody (Amersham) and then a 1:1,000-diluted avidin-alkaline phosphatase conjugate (Sigma), followed by development with the nitrophenol phosphate substrate (1 mg/ml in carbonate buffer, pH 9.6). $A_{405}$ was measured with a Titertek Multiscan ELISA reader. All assays were done at least twice with duplicate test samples. Binding to individual peptides or proteins was considered significant if the mean absorbance in test wells was twice the mean absorbance in wells coated with BSA alone and greater than the mean absorbance plus 3 standard deviations of the five lowest absorbances in peptide-coated wells. The isotype of murine antibodies binding to P70 or C-142 was determined with an ELISA subtyping kit in accordance with the manufacturer's (Sigma) instructions.

**Peptide inhibition ELISA.** In other experiments, the ability of fluid-phase peptides to inhibit antibody binding to solid-phase C-142 was determined. Preliminary experiments established that the optimal coating concentration of C-142 was 0.5 μg/ml for the inhibition assays. Peptide (0.7 mM) was incubated with MAb supernatant (1:30) or hyperimmune sera (1:200) overnight at 4°C. The mixture was then transferred to C-142-coated wells, and bound antibody was detected as described above. The percent inhibition of maximum binding for individual MAbs or sera was calculated, and inhibition of >50% was considered significant.

**MAb capture and inhibition assays.** The epitopes recognized by the MAbs were characterized with a MAb capture ELISA. The wells of a polystyrene microtiter tray (Flow) were coated for 2 h at 37°C with the gamma globulin fraction of L7 or B8 (100 μg/ml). After blocking with 3% BSA, *M. leprae* or *M. bovis* P70 (0.001 to 10 μg/ml in 1% BSA) was added for 1 h at 37°C. The bound antigen was then probed with biotinylated L7 or B8, followed by avidin-conjugated alkaline phosphatase (1:1,000) and the nitrophenol phosphate substrate. The epitopes recognized by murine antisera were also investigated with MAb inhibition assays as previously described (8). Murine anti-*M. leprae* P70 and anti-C-142 at various dilutions were incubated with C-142-coated wells prior to washing and addition of biotinylated B8. Bound B8 was detected with avidin-conjugated alkaline phosphatase.

**Estimation of MAb affinity.** The affinity of binding for MAbs B7, B8, and L7 was determined by using a two-stage ELISA procedure (11) to estimate the amount of free antibody in a mixture of antibody and antigen at equilibrium. Preliminary experiments determined the optimal concentrations for individual MAbs over the range of antigen concentrations tested. The wells of a microtiter plate were first blocked with 3% BSA for 3 h at 37°C. The three MAbs (0.7, 0.35, and 0.9 μg/ml for L7, B7, and B8, respectively) were then added to triplicate wells containing no antigen or *M. bovis* P70 at concentrations of 0.01 to 100 μg/ml in 1% BSA. After incubation overnight at room temperature, these antigen-antibody solutions (50 μl) were reacted with solid-phase *M. bovis* P70 for 3 h to estimate the concentration of free antibody at equilibrium. To confirm that the proportion of free antibody bound to immobilized P70 in the ELISA reaction was less than 10%, the mixture was transferred to a second ELISA plate to react again with solid-phase P70. Both ELISA plates were developed with a goat anti-mouse IgG-alkaline phosphate conjugate (Sigma) and the nitrophenol phosphate substrate. The amount of antibody bound to

TABLE 2. Antigen capture ELISA which demonstrates that MAbs L7 and B8 bind to separate epitopes on *M. bovis* P70[a]

| Antigen concn (μg/ml) | MAb binding[b] *to captured antigen* ($A_{405}$ units) | | | |
|---|---|---|---|---|
| | L7/L7[c] | L7/B8 | B8/L7 | B8/B8 |
| 10 | 0.084 (0.001) | 1.777 (0.191) | 1.046 (0.046) | 0.097 (0.014) |
| 1 | 0.074 (0.006) | 1.610 (0.071) | 0.733 (0.049) | 0.092 (0.015) |
| 0.1 | 0.059 (0.016) | 0.642 (0.033) | 0.341 (0.024) | 0.099 (0.016) |
| 0.01 | 0.056 (0.003) | 0.130 (0.018) | 0.167 (0.008) | 0.117 (0.022) |

[a] Binding of biotinylated detector MAb to captured antigen was determined with a streptavidin-alkaline phosphatase conjugate.
[b] Mean of triplicate wells ± the standard deviation.
[c] L7/L7, capture MAb/detector MAb.

antigen at each concentration was estimated from the antibody reactivity with and without antigen ($A_{405}$). A plot of the reciprocal of the antigen concentration against the reciprocal of the relative concentration of the bound antibody yielded a straight line, the slope of which was the dissociation constant ($K_d$) for the reaction (11).

## RESULTS

The purified recombinant C-142 fragment of *M. leprae* P70 reacted with the three MAbs and human leprosy sera in immunoblots (data not shown). A competition ELISA had previously suggested that MAbs L7 and B7 identified a different epitope from that recognized by MAb B8 (8). The discrete nature of the two epitopes was confirmed by an antigen capture ELISA in which the antigen bound by L7 reacted with B8 and vice versa (Table 2). The dissociation constants ($K_d$) of the anti-P70 MAbs after reaction with *M. bovis* P70 were $1 \times 10^{-9}$ M for L7 and $3 \times 10^{-6}$ M for B7. The $K_d$ for B8 was $<1 \times 10^{-10}$ M, which is the limit of sensitivity for this method (11).

A series of overlapping peptides (Table 1) were then used to define the relevant epitopes on C-142. Initial experiments with either MAbs or polyclonal sera showed enhanced binding of antibody to BSA-conjugated rather than native peptides, and conjugated peptides were subsequently used in solid-phase ELISAs. MAb B8 bound to peptide P8 alone (Fig. 1). This reactivity was confirmed by fluid-phase inhibition assays in which P8 caused greater than 50% inhibition of B8 binding to C-142 (Fig. 2). The sequence AEEDRKRREE

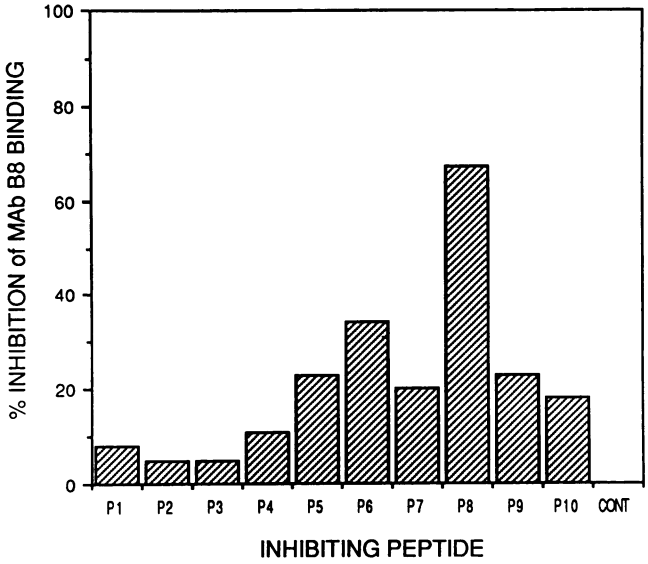


FIG. 2. Fluid-phase inhibition of the binding of antimycobacterial P70 MAb B8 to immobilized C-142 protein by peptides P1 to P10 at a concentration of 0.7 mM. CONT, control.

in the N-terminal half of P8 was shared with peptide P9, while the C-terminal half of P8, EEADVRNQAE, was present in peptide P7. However, neither P9 or P7 reacted with MAb B8. No comparable reactivity was observed with MAb L7 or B7 in either the direct-binding (Fig. 2) or the inhibition (data not shown) assay, despite strong binding of both MAbs to C-142.

The reactivity of human leprosy sera with the C-142 peptides was then examined. Patients were selected on the basis of reactivity with *M. leprae* P70 and the C-142 fragment in a solid-phase ELISA. Ten such C-142-reactive sera failed to bind significantly to native or conjugated peptides. In a minority of the cases (2 of 10), however, peptides P2 and P3 were able to inhibit binding of patient serum to immobilized C-142 (Fig. 3), suggesting a degree of reactivity with this *M. leprae*-specific sequence of the protein.

The lack of direct reactivity of leprosy sera with C-142 peptides led us to examine whether hyperimmune sera from animals immunized with *M. leprae* P70 could identify linear

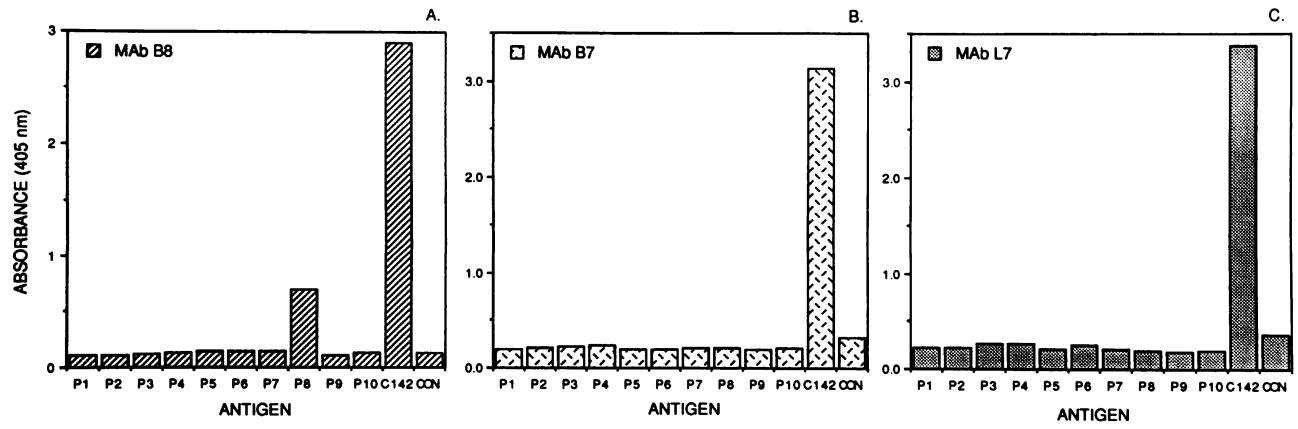


FIG. 1. Binding of MAbs B8, B7, and L7 to the C-142 fragment of *M. leprae* P70, conjugated peptides P1 to P10, or control (CON) wells coated with BSA alone in a solid-phase ELISA.

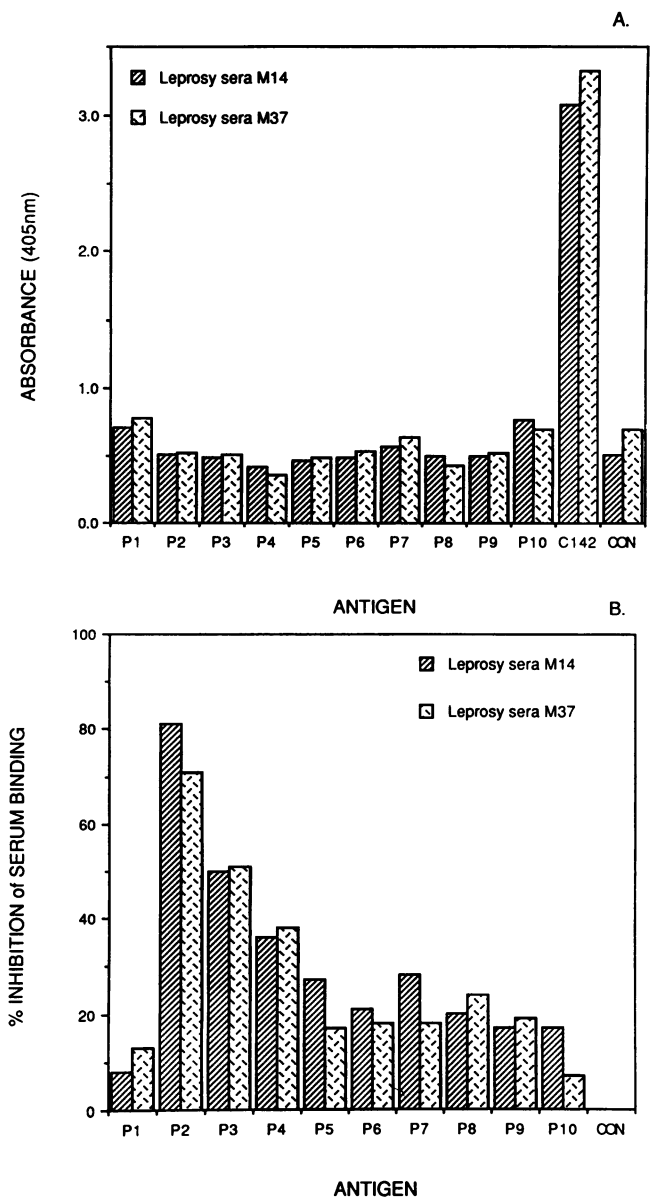


FIG. 3. (A) Binding of two C-142-reactive leprosy sera to the C-142 protein, conjugated peptides P1 to P10 and control (CON) wells coated with BSA. (B) Fluid-phase inhibition of the binding of the same two C-142-reactive leprosy sera to immobilized C-142 protein by peptides P1 to P10 at a concentration of 0.7 mM.

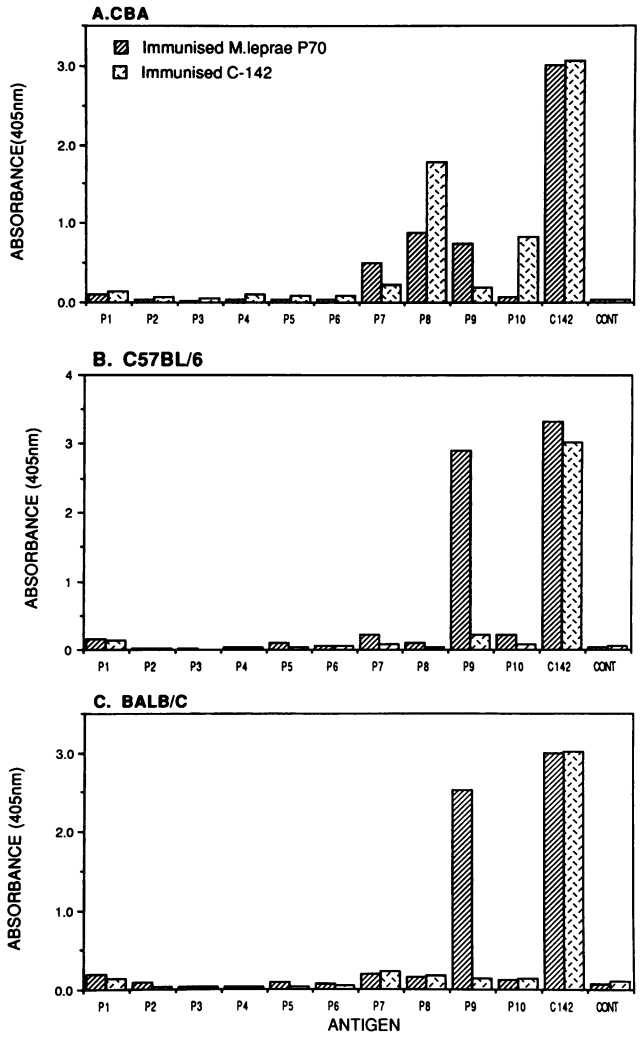


FIG. 4. Binding of murine antisera to either the C-142 protein or the conjugated peptides in a solid-phase ELISA. Three strains of mice (CBA [A], C57BL/6 [B], and BALB/C [C]) were immunized with either *M. leprae* P70 or C-142 protein in incomplete Freund's adjuvant, and the pattern of their antibody responses was determined at 21 days. CONT, control.

epitopes in the mycobacterium-specific region of the protein. Hyperimmune sera from three strains of mice following immunization with either the whole protein or the C-142 fragment were tested for reactivity with the conjugated peptides (Fig. 4). In both C57BL/6 ($H\text{-}2^b$) and BALB/c ($H\text{-}2^d$) strains, the antibody-dominant response following immunization with *M. leprae* P70 was to P9. When the mice were immunized with C-142 alone, however, the sera failed to react strongly with any of the immobilized peptides in an ELISA, despite significant binding to C-142 (Fig. 4). By comparison, sera from CBA ($H\text{-}2^k$) mice immunized with P70 reacted with three adjacent peptides, P9, P8, and P7. Following immunization with C-142 sera from $H\text{-}2^k$ mice showed strong binding to P8 and to a lesser extent to P10 (Fig. 4). The dominant isotype in the IgG response of each strain to C-142 and the whole protein was IgG1 (data not shown). These results suggest that murine antisera recognize dominant linear B-cell epitopes within the sequence between residues 487 and 532 but that there is variation in the fine specificity of antibody binding between the strains tested.

It was surprising that MAb B8 derived from BALB/c mice reacted with P8 (residues 498 to 515), but BALB/c polyclonal anti-P70 or anti-C-142 sera reacted predominantly with P9 (residues 487 to 507) (Fig. 4). We therefore examined whether the BALB/c antisera were able to inhibit the binding of biotinylated MAb B8 to C-142. Both anti-P70 and anti-C-142 antisera, but not control murine sera, caused 50% inhibition of B8 binding to C-142 at titers of 1:80 and 1:1,000, respectively. This suggests that steric hindrance caused by the binding of polyclonal antibodies to the regions neighboring residues 487 to 507 prevented the binding of MAb B8 to its epitope.

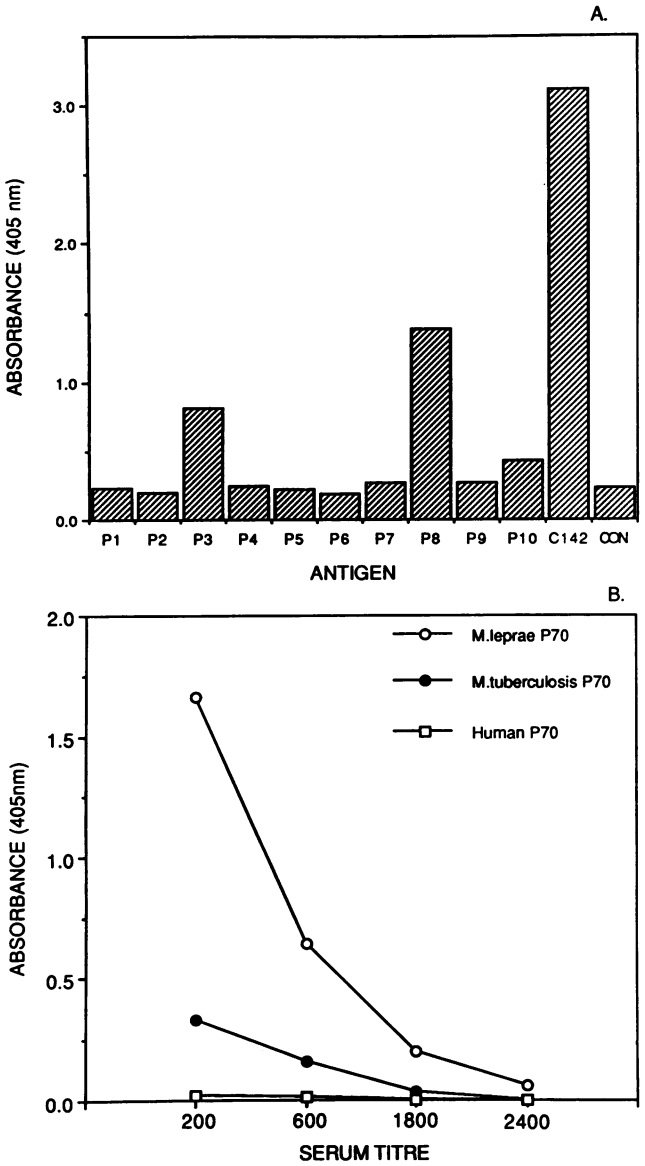


FIG. 5. (A) Binding of hyperimmune rabbit anti-*M. leprae* P70 to the C-142 protein and conjugated peptides P1 to P10 in a solid-phase ELISA. CON, control. (B) Binding of hyperimmune rabbit anti-peptide 3 serum to *M. leprae*, *M. tuberculosis*, or human P70.

Rabbit polyclonal anti-*M. leprae* P70 hyperimmune sera also showed strong reactivity with P8, but in addition identified an epitope on P3 within the *M. leprae*-specific region (Fig. 5A). To confirm this, rabbits were immunized with a P3-ovalbumin conjugate and their sera were tested with whole protein. P3-ovalbumin conjugate antisera recognized *M. leprae* P70 strongly, confirming that the P3 sequence contains a linear B-cell epitope exposed on the surface of the whole protein (Fig. 5B). There was much less reactivity with *M. tuberculosis* P70 and none with human HSP70 (Fig. 5B).

## DISCUSSION

These mapping studies with synthetic peptides have defined continuous antibody epitopes on *M. leprae* P70 that are recognized by MAb B8 and hyperimmune murine and rabbit antisera. The linear epitopes are chiefly within the *M. leprae* P70 sequence between residues 487 and 532, which is shared by *M. leprae* and *M. tuberculosis* but significantly differs from the similar region in the human and murine HSP70 proteins (14, 15). The epitope defined by MAb B8 is in the central region of P8 (RKRREEADVR), as the antibody failed to react with the adjacent peptides P7 and P9, which overlap P8 by 10 and 7 residues, respectively (Table 1). By contrast, the separate epitope recognized by MAbs L7 and B8 (Table 2) is conformational in nature, as neither L7 nor B8 reacted with the peptides, despite strong reactivity with both soluble *M. leprae* P70 and C-142 (Fig. 1). As L7 and B8 react with both *M. tuberculosis* and *M. leprae* P70, this epitope must be within the C-terminal region from Glu-478 to Ala-552, as the protein sequences diverge completely from Ala-552 (18). While a number of anti-*M. leprae* MAbs have identified linear epitopes in the 65-, 36-, and 28-kDa proteins, other MAbs clearly react with conformational determinants (2, 14, 16). For example, *M. leprae*-specific MAb L5 (6) reacted with a 48-residue peptide from the 18-kDa protein but not with shorter 20-mers from the same region (9).

The humoral response of leprosy patients is directed to the C-terminal region of *M. leprae* HSP70 (8), but we were unable to detect direct binding of sera with conjugated or unconjugated peptides, despite selection of sera for strong reactivity to the C-142 fragment and P70. Therefore, these sera reacted with conformational determinants in the C-terminal 142 residues. Recent studies of B-cell epitopes on other mycobacterial proteins have also failed to detect linear epitopes on the 10-, 36-, and 65-kDa proteins with sera from infected patients (16, 26, 27). Furthermore, in the case of each protein, sera which inhibited MAb binding to the protein failed to bind directly to peptides containing the MAb-defined linear epitope. This indicates that inhibition was due to steric hindrance caused by binding of the patient sera to a neighboring conformational epitope. By contrast, polyclonal sera from mice and rabbits immunized with *M. leprae* P70 or C-142 reacted strongly with a limited number of linear epitopes in the C-terminal fragment of the protein (Fig. 4 and 5). The murine antisera identified epitopes in the shared hydrophilic region between residues 487 and 532 (peptides P7 to P9) which contained the MAb-defined epitope. Rabbit hyperimmune anti-P70 sera identified another epitope, between residues 567 and 591, in the *M. leprae*-specific C-terminal 70 residues, in addition to the epitope in the sequence between residues 498 and 515 shared with *M. tuberculosis* (Fig. 5). Rabbit antisera raised against P3 (sequence between residues 567 and 591) reacted strongly with *M. leprae* P70 and not with human HSP70, confirming that the *M. leprae*-specific region of P70 can generate a mycobacterium-specific antibody response without cross-reactivity to eukaryotic HSP70.

Various factors could influence the difference in the epitopes recognized by patient sera and hyperimmune sera. (i) Protein antigens presented to the immune system during mycobacterial infections may differ in form from those used for immunization. The purified recombinant proteins may contain partially denatured components which elicit an antibody response to a linear determinant not normally exposed on native protein. The importance of antigen conformation is demonstrated by the difference in the responses of C57BL/6 and BALB/c mice to the whole *M. leprae* P70 protein, which stimulated a response to a linear epitope on peptide P9, and the antibody response to fragment C-142, which recognized only conformational epitopes. (ii) The

affinity of binding of hyperimmune sera to the immunizing antigen may be greater than that of patient sera. Polyclonal antisera typically have a 5,000-fold higher affinity for native protein than for reactive peptides within the protein (22). This would favor the identification of linear peptide epitopes by hyperimmune sera or high-affinity MAbs such as B8. Although the two-stage ELISA provided only an estimate of antibody affinity, it demonstrated the hierarchy of binding to P70 for these MAbs and it was the highest affinity MAb, B8, which defined a linear determinant. (iii) Antibody responses may be affected by differences in species and major histocompatibility complex haplotype. This was reflected in the difference between the murine and rabbit antibody responses to the linear determinant on peptide P3. Furthermore, there were differences in the fine specificities of antibody binding to the peptide determinants among the three murine strains (Fig. 4). This is in keeping with the influence of the *H-2* locus on the murine antibody responses to other mycobacterial proteins, including the *M. leprae* 18- and 65-kDa proteins (4, 9).

In summary, the mycobacterium-specific antibody response in leprosy patients is directed at conformational epitopes in the C-terminal region of *M. leprae* P70. This is in keeping with the available X-ray crystallographic characterization of antibody-binding sites, which shows them to be composed of discontinuous sequences involving two to five surface loops of a protein (17). This highlights the intrinsic limitation of using linear peptides as tools for epitope mapping with human sera and as reagents for specific serodiagnostic tests. Nevertheless, synthetic peptides proved of value in mapping the determinants recognized by MAbs and hyperimmune polyclonal antisera (13) and confirmed the potential of these determinants to generate mycobacterium-specific B-cell responses.

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