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The Variable C-Terminal Region of the *Mycobacterium leprae* 70-Kilodalton Heat Shock Protein Is the Target for Humoral Immune Responses

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The 70-kDa heat shock protein of *Mycobacterium leprae* has a high degree of homology with the human hsp70 protein, yet it still elicits T-lymphocyte responses in subjects infected with *M. leprae* or vaccinated with the related *Mycobacterium bovis* BCG. We examined the serological responses to this protein by using recombinant protein fragments expressed from mutants with deletions of the *M. leprae* p70 gene. Monoclonal antibodies raised against either *M. bovis* or *M. leprae* p70 reacted with the C-terminal fragments but not the N-terminal fragments in a solid-phase enzyme-linked immunosorbent assay and an immunoblot assay. Inhibition enzyme-linked immunosorbent assays confirmed that two separate epitopes were defined by these monoclonal antibodies. Murine polyclonal sera also showed stronger binding to the C-terminal fragments. Sera from 33 and 48% of lepromatous leprosy patients reacted with *M. leprae* and *M. bovis* p70. This reactivity was mycobacterium specific, since few sera from control subjects in the same leprosy-endemic region were seropositive. The levels of anti-mycobacterial hsp70 antibodies were higher in patients with lepromatous leprosy than in those with tuberculoid leprosy or tuberculosis. The reactivity of sera from patients with leprosy was maximal with the C-terminal fragments. Therefore the C-terminal portion of *M. leprae* hsp70, which includes the region of maximum divergence from human hsp70, is the major target for the humoral immune response to the protein.

Leprosy remains an important public health problem worldwide, with an estimated 10 to 12 million patients affected and 1.32 billion people at significant risk of contracting the disease (27). Cell-mediated immune responses to the causative organism, *Mycobacterium leprae*, are protective in the majority of infected individuals but are also responsible for the immunopathology characteristic of the tuberculoid form of the disease (5). Patients with the lepromatous form of the disease have evidence of deficient cellular responses but strong antibody responses to antigens of *M. leprae* (19). The characterization of these antigens is essential for understanding the complex regulation of immune responses across the leprosy spectrum and for the rational development of vaccines and diagnostic agents. An increasing number of mycobacterial proteins have been identified with monoclonal antibodies (MAbs) (14), including members of the heat shock protein (HSP) family. HSPs or stress proteins are widely conserved in prokaryotes and eukaryotes (23) and have essential physiological functions as molecular chaperonins in the folding and translocation of proteins (3, 12, 21) and in protecting proteins from denaturation during cellular stress (16, 22). Despite the conservation of these proteins, HSPs from a wide range of bacteria and parasites have been identified as targets of host immune responses (35).

The 65- and 70-kDa proteins of *M. leprae* were initially identified as HSPs because of their sequence homology with other members of the hsp60 and hsp70 families, respectively (17, 38). Later the related *Mycobacterium tuberculosis* 65- and 70-kDa proteins were shown to respond to heat stress (22), and the *M. bovis* 70-kDa protein was shown to have the biochemical properties of ATPase activity and autophosphorylation that are typical of the hsp70 family (29). The *M. leprae* 65-kDa protein stimulates significant T-cell and antibody responses in leprosy patients (26, 37), and numerous B- and T-cell epitopes on the protein have been defined (9, 28). Studies with affinity-purified *M. bovis* 70-kDa protein have demonstrated that the mycobacterial hsp70 is recognized by *Mycobacterium bovis* BCG vaccinees and leprosy patients and their contacts (1, 7). The complete gene for the *M. leprae* hsp70 was recently cloned, and the expressed recombinant protein was shown to elicit T-cell responses in immune subjects (25). In this study we report the serological responses to mycobacterial hsp70 in leprosy patients and hyperimmune mice and the use of recombinant fragments of *M. leprae* hsp70 (ML rP70) to analyze the regions of the protein involved in antibody binding. This study demonstrates that the C-terminal region of the protein is the major target for human and murine antibody responses.

MATERIALS AND METHODS

Construction of plasmids containing *M. leprae* P70 gene fragments. Fragments of the gene encoding the *M. leprae* 70-kDa protein were cloned into the expression plasmids pGEX-1N, pGEX-2T, and pGEX-3X by ligating the fragments in reading frame to DNA encoding the carboxyl (C)-terminal portion of glutathione-*S*-transferase (GST) from *Schistosoma japonicum* (36). The gene fragments were prepared, either by using the polymerase chain reaction with restriction enzyme sites engineered for cloning into pGEX-2T or by digesting at restriction sites already present within the gene (Fig. 1). Gene fragments not containing the stop codon were ligated with a pGEX vector in reading

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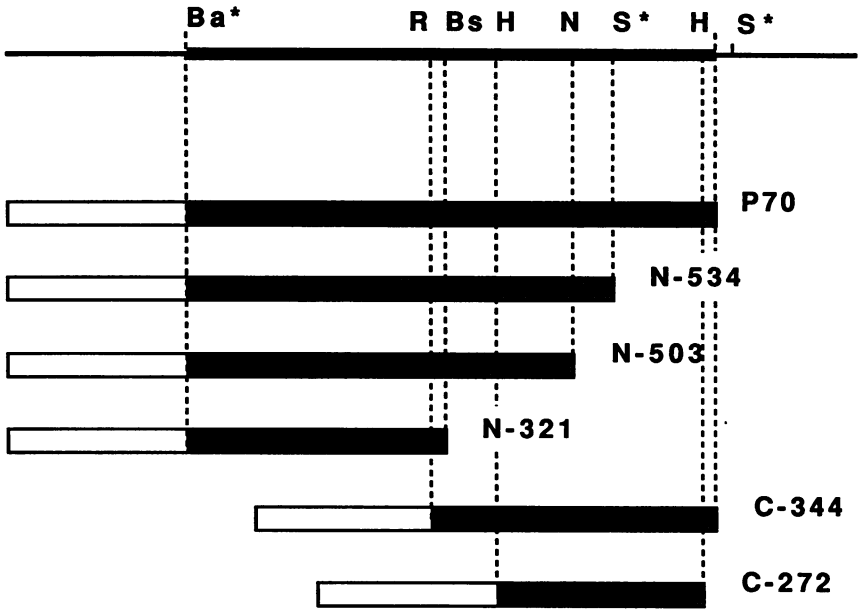

FIG. 1. Map of recombinant *M. leprae* P70, composed of 621 amino acids; three N-terminal fragments extending 534, 503, and 321 amino acids from the N terminus; and two C-terminal fragments, one extending 344 amino acids from the C terminus and one of 272 amino acids extending to within 10 residues of the C terminus. Each protein was expressed as a fusion protein attached to the C-terminal portion of GST. A map of the gene for the whole *M. leprae* P70 shows the restriction sites used for construction of the plasmids. The restriction enzymes used were *Bam*HI (Ba), *Bst*EII (Bs), *Hin*dII (H), *Nru*I (N), *Sma*I (S), and *Eco*RI (R). The sites engineered by using the polymerase chain reaction are indicated (*).

frame with a stop codon several base pairs downstream of the cloning sites.

Plasmids pGT-503 and pGT-321, containing 1,511 and 962 bp, respectively, were derived from pGEX70, which contains the whole P70 reading frame in the vector pGEX-2T (25). Plasmid pGEX70 was digested with *Nru*I (blunt ended) and *Sma*I for pGT-503 and with *Bst*EII and *Sma*I for pGT-321. After removal of the smaller fragments, the plasmid-containing fragments were self-ligated. The polymerase chain reaction was used to generate a 1,613-bp fragment from pGEX70 with a 5′ *Bam*HI site and a 3′ *Sma*I site. This fragment was cloned into pGEX-2T to produce plasmid pGT-534. Two fragments were derived from the clone JKL2 (17), which contained approximately 1 kb of DNA encoding the C-terminal portion of *M. leprae* P70. The clone was digested with *Eco*RI, and the entire JKL2 insert was cloned into pGEX-2N to produce pGN-344. Plasmid pGX-272 was constructed from an 800-bp *Hin*dII end-filled fragment ligated with *Sma*I-digested pGEX-3X. A map of the clones is shown in Fig. 1.

Purification of *M. leprae* recombinant proteins. The gene fragments were expressed in *Escherichia coli* MC1061, and the recombinant proteins were purified as fusion proteins (ML rP70f and fragments of ML rP70) by the method of Smith and Johnson (36), with modifications. Production of the fusion protein was induced during log-phase growth of *E. coli* by incubation for 3 h at 37°C in the presence of 0.1 mM isopropyl-β-D-thiogalactopyranoside (Sigma, St. Louis, Mo.). The pelleted bacteria were sonicated in 10 ml of phosphate-buffered saline (PBS; 0.01 M sodium phosphate–0.15 M sodium chloride) containing 1% Triton X-100 per liter of original culture. After centrifugation at 10,000 × *g* for 5 min, the fusion protein was purified from the supernatant by affinity chromatography with glutathione agarose (Sigma), eluted with 5 mM glutathione (Sigma), dialyzed against 0.1-strength PBS, and lyophilized. In addition, *M. leprae* hsp70 was produced as a soluble protein (ML rP70c) by cleaving it directly from GST with thrombin as described by Gearing et al. (18). GST was purified with *E. coli* transformed with pGEX-2T and used as a control antigen.

***M. bovis* P70 antigen.** The *M. bovis* P70 antigen (MB P70) was purified from culture filtrate of *M. bovis* A5, kindly provided by P. Wood, by using affinity chromatography with MAb L-7 as previously described (7). Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed a dominant band of 70 kDa and faint bands at 30 and 36 kDa, all of which reacted with the L-7 MAb.

Human sera. Sera were collected from untreated leprosy patients from leprosy clinics in Nepal (50 samples) and Sydney (38 samples) and from normal subjects in Sydney (190 samples) and in Nepal (50 samples). The leprosy patients were classified according to clinical and bacteriological criteria (31). A leprosy serum pool was prepared from the sera of 10 lepromatous leprosy patients. All sera were stored at −20°C in 0.02% sodium azide until use.

Murine antisera and MAbs. BALB/c mice were immunized at 0 and 7 days with 20 or 50 μg of MB P70 or ML rP70c in incomplete Freund adjuvant (CSL, Melbourne, Australia) and again at 14 days with antigen in PBS. Sera were collected 7 days later. Mice used for the production of MAbs were boosted intravenously with antigen in PBS 3 days before fusion. Spleen cells were mixed with myeloma cells (P3-NS-1-Ag4-1 or Sp-2/0-Ag-14) at a ratio of 10:1 in the presence of polyethylene glycol (PEG 4000 [Merck, Darstadt, Germany] or PEG 1000 [Ajax, Sydney, Australia]). Cells were cultured as described elsewhere (10), and supernatants were screened by a solid-phase enzyme-linked immunosorbent assay (ELISA) with MB P70 or ML rP70c at 1 μg/ml as the antigen. Hybridomas producing antibodies of the desired specificity were cloned by limiting dilution three

times. Two anti-MB P70 MAbs (B-7, B-8) were produced by this method. Previously, two anti-ML P70 MAbs (L-7, L-27) had been produced by immunization with a sonic extract of whole *M. leprae* cells (8). The isotype of the MAb was determined with a mouse hybridoma subtyping ELISA kit (Boehringer Mannheim, Germany). The pattern of MAb binding to mycobacteria was examined by ELISA and immunoblotting with sonic extracts of *M. leprae* and *M. bovis*.

ELISAs. Polystyrene 96-well microtiter plates (Linbro; ICN-Flow, Sydney, Australia) were coated with antigen in PBS overnight at 37°C. The optimal antigen concentrations of MB P70 and ML rP70c in the screening assays of human sera were determined by antigen titration to be 5 and 10 μg/ml, respectively. The recombinant fragments of ML rP70 were used at a concentration of 10 μg/ml. Plates were blocked with 3% bovine serum albumin (BSA; fraction V; Miles Laboratories, Naperville Ill.) in PBS and then incubated for 1 h with the first antibody, which consisted of MAb supernatant, mouse serum diluted 1/200 in 1% BSA, or human serum diluted 1/100 in 10% normal goat serum and 1% BSA. After three washes with PBS, the second antibodies (alkaline phosphatase-conjugated anti-mouse immunoglobulin G [IgG] or anti-human IgG [Sigma], diluted 1/1,000 in 1% BSA) were added. Wells were then washed three times with PBS and once with substrate buffer before incubation with *n*-nitrophenyl phosphate substrate (Sigma) for 30 min. The A_{405}s were measured with a Titretek Multiscan ELISA reader (Flow).

SDS-PAGE and immunoblotting. The P70-derived proteins were boiled in reducing buffer (0.1 M Tris [pH 6], 8% glycerol, 1 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue) for 10 min and then loaded on a 10% SDS–PAGE gel. Proteins separated by electrophoresis were either stained with 0.05% Coomassie blue (10 μg per track) or transferred to nitrocellulose (Schleicher and Schuell, Dassel, Germany) (2 μg per track) in a Bio-Rad Mini Protean II transfer cell (Bio-Rad, Richmond, Calif.) at 250 mA for 1 h. After overnight incubation in 1% BSA at 4°C, the nitrocellulose strips were incubated in neat L-7 culture supernatant or antiserum diluted 1:150 with 1% BSA in Tris-buffered saline (50 mM Tris, 0.15 M NaCl, 5 mM EDTA [pH 7.1]) plus 0.05% Tween 20 for 2 h at room temperature. Biotinylated goat anti-mouse or goat anti-human IgG and streptavidin-biotinylated horseradish peroxidase complex (Amersham International, Amersham, United Kingdom) were used as the second antibodies according to the instructions of the manufacturer. The color reaction was developed by adding peroxidase substrate (1 mg of α-chloronaphthol [Sigma] per ml) in the presence of 0.025% hydrogen peroxide.

RESULTS

Production of recombinant antigens. The constructs containing fragments of the *M. leprae* P70 gene encoded proteins containing the N-terminal 534, 503, and 321 amino acids and the C-terminal 344 and 272 amino acids (Fig. 1). After expression in *E. coli*, the resulting fusion proteins were affinity purified by binding to glutathione-agarose (Fig. 2A). The principal protein bands had relative molecular masses corresponding with those predicted for fusion proteins containing the additional 26-kDa GST fragment. Some breakdown of P70 fragments was evident on analysis by SDS-PAGE. A band of 26 kDa corresponding to free GST was present in gels of some fragments (Fig. 2A, tracks b through d), suggesting either that GST was synthesized in addition to the fusion protein or that some fusion proteins were cleaving near the junction of P70 and GST.

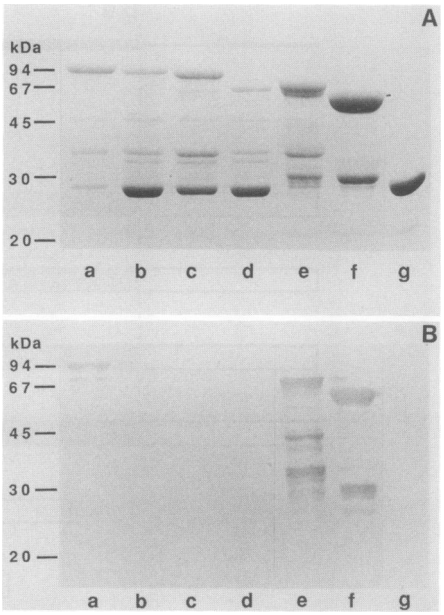

FIG. 2. SDS-PAGE analysis of *M. leprae* rP70f and recombinant fragments of the protein stained for protein with Coomassie blue (A) and probed with MAb L-7 in immunoblots (B). The tracks contain the *M. leprae* recombinant 70-kDa protein (a), N-534 (b), N-503 (c), N-321 (d), C-344 (e), C-272 (f), and GST (g). Molecular mass markers are shown on the left.

Specificity of MAbs. Anti-MB P70 MAbs (B-7, B-8) were generated after immunization of mice with the affinity-purified MB P70. Both reacted in immunoblots with P70 from *M. leprae* or *M. bovis*, as did MAbs L-7 and L-27 obtained from previous fusions of spleen cells from mice immunized with *M. leprae* sonic extracts (8) (Fig. 3). Cross-reactivity with other mycobacteria was confirmed in ELISAs with a variety of mycobacterial sonic extracts as antigens. The anti-mycobacterial MAbs did not react with *E. coli* lysates or with recombinant human hsp70 (data not shown).

Inhibition assays with the four anti-p70 MAbs demonstrated that they defined two separate epitopes. MAbs L-7 and L-27 inhibited the binding of MAbs L-7, L-27, and B-7 to MB P70 or ML rP70c as antigens. MAb B-7 blocked the binding of itself at high concentrations of inhibiting antibody but failed to block the binding of other MAbs to either antigen (Fig. 4A). This appeared to be due to the low affinity of MAb B-7 for MB P70 and ML rP70c (data not shown). By contrast, B-8 blocked the binding of itself but not that of L-7, L-27, or B-7 to the two antigens (Fig. 4B). The other three MAbs did not inhibit the binding of B-8. In other words, B-8 defines a separate cross-reactive epitope that is present on both MB P70 and ML rP70c. Despite the large number of fusions performed, no *M. leprae*- or *M. bovis*-specific epitopes were identified.

Murine responses to *M. leprae* fragments. Immunoblots of the recombinant fragments probed with anti-*M. leprae* MAb L-7 showed that this MAb reacted with whole ML rP70c and the C-terminal fragments C-344 and C-272 (Fig. 2B). When ELISAs were performed with L-7 and the other three MAbs, each bound to the C-terminal fragment but not the N-terminal fragment of ML rP70 (Fig. 5). This suggests that the two epitopes recognized by these antibodies lie within the por-

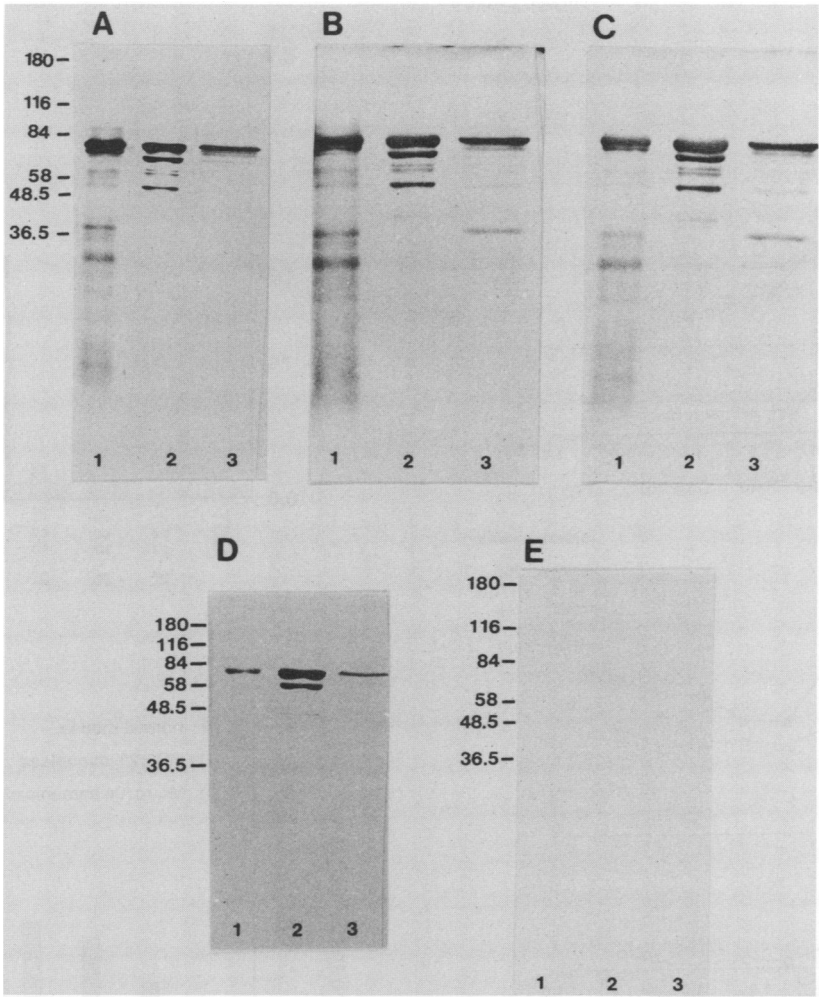

FIG. 3. Binding of anti-mycobacterial P70 MAbs to *M. bovis* P70 (tracks 1), *M. leprae* rP70c (tracks 2), and *M. bovis* BCG sonic extract (tracks 3) in immunoblots of proteins separated by SDS-PAGE. The MAbs include L-7 (A), L-27 (B), B-8 (C), B-7 (D), and control IgG1 MAb K-1-21 directed against human kappa light chain (E).

tion of the C-terminal fragments that does not overlap with fragment N-534 (Fig. 1).

When the murine polyclonal anti-p70 response was examined, sera from mice immunized with either ML rP70c or MB P70 showed stronger binding to the C-terminal fragment than to the N-terminal fragment of ML rP70 (Fig. 5B). The binding to all fragments was significantly greater than that to the control recombinant antigen, GST, purified from *E. coli* by the same procedure.

Human responses to ML fragments. The human antibody responses to ML rP70c and MB P70 were examined by using the ELISA with sera from control subjects and from patients with leprosy or tuberculosis. Few sera from control subjects in leprosy-endemic (50 samples) or nonendemic (190 samples) countries contained anti-p70 antibodies (Table 1). There was a differential reactivity of patient sera with *M. leprae* or *M. bovis* hsp70. One-third of sera from borderline lepromatous and lepromatous leprosy patients had IgG anti-ML rP70c antibodies, and 48% had IgG anti-MB P70 antibodies. These two groups did not overlap completely; antibodies to both antigens were detected in only 12% of borderline lepromatous and lepromatous leprosy patients. Fewer borderline tuberculoid and tuberculoid leprosy patients had anti-mycobacterial hsp70 antibodies. An intermediate proportion of tuberculosis patients (19 to 24%) were seropositive to either antigen (Table 1), and 14% had IgG antibodies to both MB P70 and ML rP70c. When compared with those in control subjects, the mean levels of anti-ML rP70c IgG were significantly higher in borderline lepromatous and lepromatous leprosy patients ($P < 0.0005$), in borderline tuberculoid and tuberculoid leprosy patients ($P < 0.0005$), and in tuberculosis patients ($P < 0.005$). A similar pattern was seen in the levels of anti-MB P70 IgG (Table 1).

Sera from lepromatous leprosy patients with high levels of anti-ML rP70c antibodies were examined for binding to ML rP70c, GST, and the recombinant fragments of ML rP70. The absorbance for each antigen was expressed as a percentage of the response of that subject to ML rP70c. There was significantly higher binding to the C-terminal fragments of ML rP70 than to the N-terminal fragments ($P < 0.05$, paired t test) (Fig. 6). Lepromatous leprosy sera also reacted with the C-terminal fragments rather than with the N-terminal fragments in immunoblots (Fig. 7).

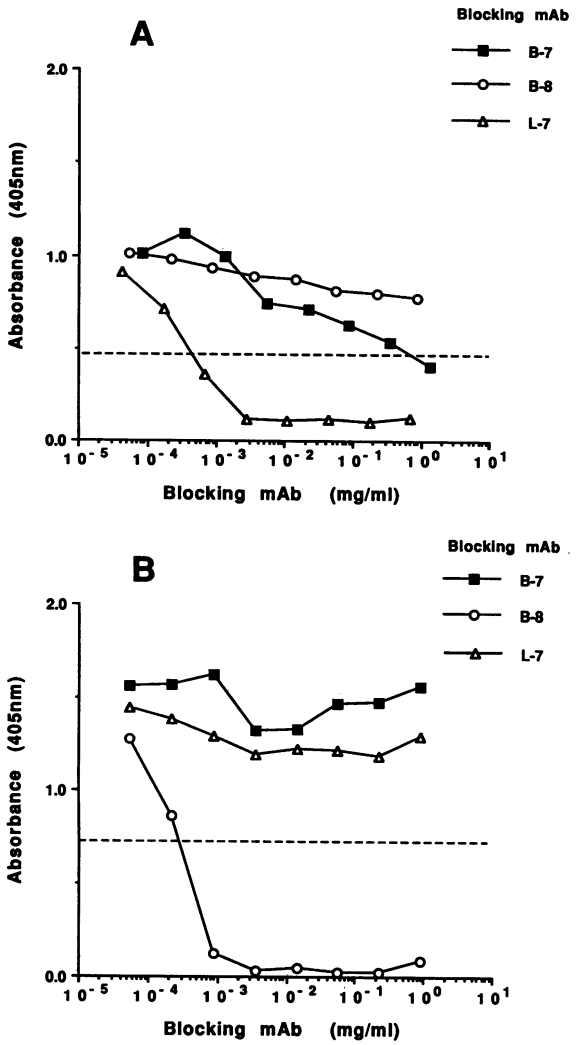

FIG. 4. Competitive inhibition assays in which the binding of MAbs B-7 (A) and B-8 (B) to *M. leprae* rP70 in a solid-phase ELISA is inhibited by blocking MAbs L-7, B-7, and B-8. The dotted lines indicate 50% of maximum binding of MAbs B-7 (A) and B-8 (B) to the antigen.

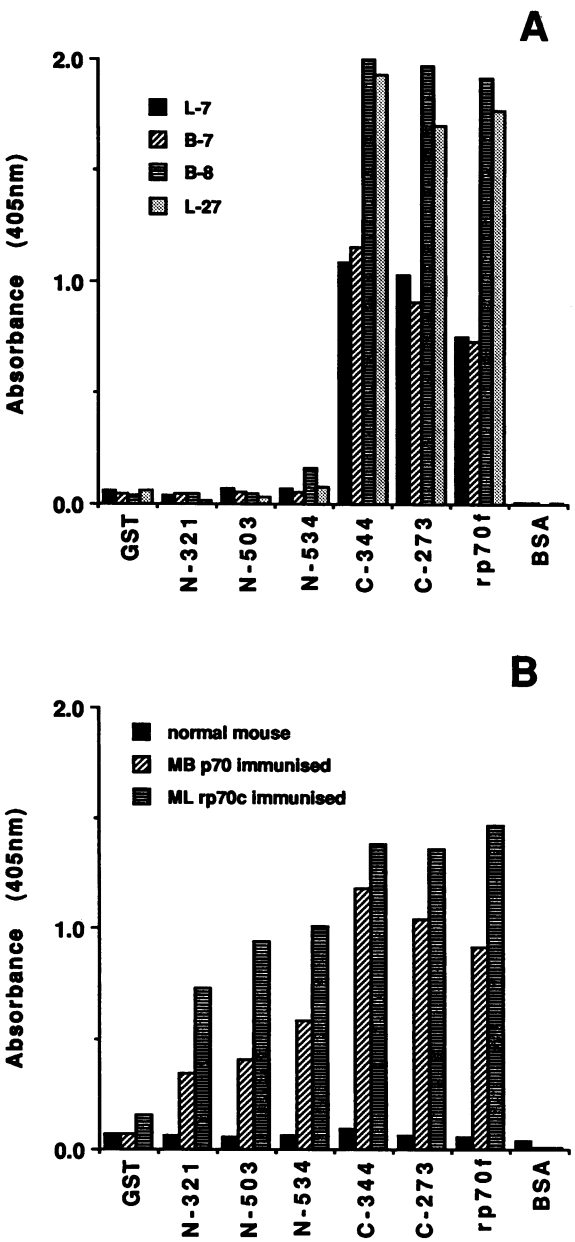

FIG. 5. ELISAs in which murine MAb (A) and murine polyclonal antisera (B) bind to *M. leprae* rP70f, recombinant fragments of rP70f, GST, and BSA. The murine MAbs are anti-*M. leprae* L-7 and L-8 and anti-*M. bovis* B-7 and B-8. The murine sera are from mice immunized with *M. leprae* rP70c, MB P70, or PBS.

DISCUSSION

M. leprae hsp70 is a target for cellular immune responses in subjects exposed to mycobacterial infections (1, 25, 32). This study confirms that a proportion of leprosy and tuberculosis patients (Table 1) and immunized animals also mount an antibody response to the protein despite the strong homology of mycobacterial hsp70 with mammalian hsp70. The human seroreactivity was greater in lepromatous leprosy patients than in tuberculoid leprosy or tuberculosis patients, a pattern observed with other mycobacterial protein antigens (26, 32). The panel of *M. leprae* rP70 recombinant fragments permitted analysis of the regions of the protein stimulating this antibody response. The murine antimycobacterial hsp70 MAbs bound to the C-terminal fragments but not to the N-terminal fragments of ML rP70 (Fig. 2B and 5), suggesting that the C-terminal region contains both of the epitopes identified by the four MAbs (Fig. 4). In addition, murine polyclonal anti-hsp70 antisera showed preferential binding to the C-terminal fragments (Fig. 5B), although repeated immunization of mice with *M. leprae* hsp70 did elicit an antibody response to all fragments, including N-321. Sera from lepromatous leprosy patients also preferentially recognized the C-terminal fragments (Fig. 7) but showed no significant binding to the N-321 fragment (Fig. 6). The difference in reactivity of murine and human sera with the N-321 fragment may be due to regions of divergence between the murine and human hsp70 proteins within this N-terminal portion (2).

Sequence analysis of the *M. leprae* P70 and human hsp70 proteins demonstrates greater conservation in the N-terminal region. Overall, there is 47% identity at the amino acid

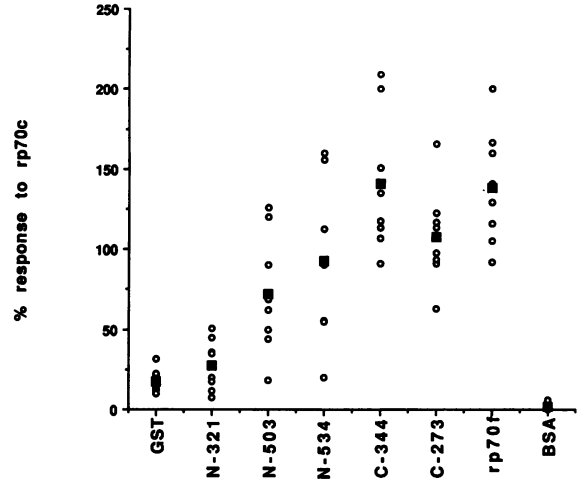

FIG. 6. ELISAs in which sera from eight positive leprosy patients (○) bind to *M. leprae* rP70f, recombinant fragments of the protein, GST, and BSA. The responses are the proportion of maximum binding of the respective sera to *M. leprae* rP70c. The mean response for each antigen is shown (■).

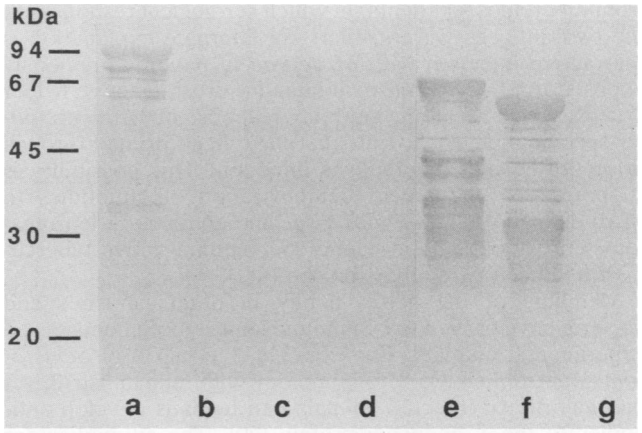

FIG. 7. Immunoblot of *M. leprae* rP70 and recombinant fragments of the protein separated by SDS-PAGE and probed with serum from a lepromatous leprosy patient. The tracks contain *M. leprae* rP70 (a), N-534 (b), N-503 (c), N321 (d), C-344 (e), C-272 (f), and GST (g). Molecular mass markers are shown on the left.

level; this ranges from 48% in fragment N-321 to 43% in fragment C-272 (25). However, the divergence is greatest in the C-terminal 100 amino acids, where only 17% of residues are conserved. This region may be responsible for the preferential reactivity of C-272 and C-344 with anti-mycobacterial P70 antibodies (Fig. 7). This has been confirmed by our more recent observation that a C-terminal 142-amino-acid fragment binds both human lepromatous leprosy sera and the four murine MAbs (28a). The sequence conservation in the N-terminal portions of hsp70s from various species appears to be necessary to preserve the ATPase function of the molecule. The ATP binding site of the related bovine hsp70 is located in the proximal half of the protein (15), whereas autophosphorylation of *E. coli* hsp70 requires the N-terminal 60 kDa of the protein (11). *M. bovis* hsp70 has retained these properties, both of which can be stimulated by the binding of hsp70 to other proteins, consistent with the role of hsp70 in facilitating protein folding (3, 29).

Comparison of the sequences for the *M. leprae* (25) and *M. tuberculosis* (21a) hsp70s demonstrates that, although there is 83% identity between the two proteins, a similar pattern of divergence in the C-terminal 100 amino acids occurs. The complete sequence of *M. bovis* hsp70 is not available (17), but it is likely to resemble hsp70 from the closely related *M. tuberculosis* rather than that from *M. leprae*. The pattern of homology differs from that for the 65-kDa HSP, in which the limited 5% divergence between the *M. leprae* gene and the identical *M. tuberculosis* and *M. bovis* genes is scattered across the gene rather than being concentrated in one region. The differences in the hsp70 C-terminal region between mycobacterial species may be responsible for the differential responses to the MB P70 and ML rP70c. For example, immunization of mice with either mycobacterial hsp70 resulted in higher antibody levels to the immunizing protein (Fig. 5B). We have observed a similar difference in the murine cellular proliferative responses to MB P70 and ML rP70c antigens. Immunization with ML rP70c in a range of mouse strains stimulated a greater response to itself than to MB P70 (unpublished observations). This suggests that the nonconserved region of *M. leprae* hsp70 contains T-cell epitopes as well as B-cell epitopes.

Surprisingly, a higher proportion of lepromatous leprosy patients were seropositive with MB P70 than with ML rP70c (Table 1). This may have been due to differences in contaminants between the native *M. bovis* and recombinant *M. leprae* antigen preparations. This was not observed with sera

TABLE 1. IgG antibody responses of control subjects and leprosy and tuberculosis patients to *M. bovis* P70 and *M. leprae* rP70[a]

Patient group (*n*)	*M. bovis* P70		*M. leprae* rP70	
	% Seropositive	Antibody level (SEM)	% Seropositive	Antibody level (SEM)
Nonendemic controls (190)	2	0.045 (0.003)	2	0.026 (0.002)
Endemic controls (50)	2	0.043 (0.006)	0	0.026 (0.004)
LL/BL leprosy (42)	48	0.470[b] (0.073)	33	0.296[b] (0.046)
BT/TT leprosy (36)	11	0.116[b] (0.019)	8	0.056[b] (0.009)
Tuberculosis (21)	19	0.124[c] (0.021)	24	0.150[b] (0.025)

[a] Antibody levels were considered positive if the levels were greater than the mean plus three standard deviations of the IgG anti-p70 antibody levels in control subjects. Antibody levels were the means of the absorbance values (A_{405}s) for individuals in each patient group. The ranges of antibody levels in lepromatous and borderline lepromatous (LL/BL) and borderline tuberculoid and tuberculoid (BT/TT) leprosy patients and tuberculosis patients were significantly different from those in control subjects.

[b] $P < 0.0005$.

[c] $P < 0.005$.

from tuberculosis patients, in which serological responses to the two antigens were similar. An alternate explanation is that active infection with *M. leprae* in patients previously exposed to *M. tuberculosis* stimulates cross-reactive helper T cells, which recall the antibody response of *M. tuberculosis*-specific memory B cells that have been primed through prior self-healing tuberculosis infection. This possibility is supported by the recent demonstration of antibodies to MAb-defined *M. tuberculosis*-specific epitopes in lepromatous leprosy patients in whom concomitant active tuberculosis had been carefully excluded (6).

Members of the hsp70 family in other parasites and bacteria have proved to be immunogenic in both humans and experimental animals. For example, the hsp70 of *Schistosoma mansoni* shows 85% homology with human hsp70, yet the majority of infected animals and humans develop antibodies to the *S. mansoni* protein (20). The antibodies are directed toward nonconserved sequences of the protein and can distinguish between infection with *S. mansoni* and that with *S. japonicum* (33). Antibodies produced against malarial hsp70 proteins are directed mainly at nonconserved epitopes (30), although a minor component also reacts with human hsp70 (24). Infection with *Brugia malayi* stimulates anti-hsp70 antibodies that react predominantly with epitopes specific to the *Brugia* protein, but there is some cross-reactivity with hsp70 molecules from *S. mansoni* and *Plasmodium falciparum* (34). A 75-kDa protective antigen of *Chlamydia trachomatis* has also been identified as a member of the hsp70 family (13), and human antibodies react primarily with nonconserved epitopes of the protein (4). Therefore, members of the hsp70 family from a wide range of parasites and bacteria are strong B-cell antigens, stimulating antibodies directed mainly toward the nonconserved regions of the protein. There is a variable degree of cross-reactivity with hsp70 proteins of other species and a minimal response to human hsp70, consistent with the need to preserve self-tolerance. In the case of *M. leprae* hsp70, the dominant antibody response is directed to the C-terminal region. Precise mapping of T- and B-cell epitopes with synthetic peptides will resolve whether there are *M. leprae*-specific epitopes on this region of the molecule.

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