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Protective Efficacy of a 62-Kilodalton Antigen, HIS-62, from the Cell Wall and Cell Membrane of *Histoplasma capsulatum* Yeast Cells

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We reported previously that a detergent extract of the cell wall and cell membrane of *Histoplasma capsulatum* yeast cells contains antigens recognized by T cells. In T-cell immunoblot analysis, a region encompassing 62 kDa was stimulatory for an *H. capsulatum*-reactive T-cell line and T-cell clones derived from C57BL/6 mice. In this study, we isolated a 62-kDa band, termed HIS-62, from electrophoresed cell wall and cell membrane of *H. capsulatum* yeast cells and examined its antigenicity and immunogenicity. C57BL/6, BALB/c, and CBA/J mice that were immunized with viable *H. capsulatum* yeast cells mounted a delayed-type hypersensitivity response to HIS-62 that was stronger than that of normal controls. Spleen cells from each strain of mouse immunized with viable yeast cells proliferated vigorously in response to HIS-62; conversely, splenocytes from control animals did not recognize this antigen. A T-cell line and 5 of 5 T-cell clones from C57BL/6 mice, 10 of 15 BALB/c T-cell hybridomas, and 8 of 12 CBA/J T-cell hybridomas recognized HIS-62. A cutaneous delayed-type hypersensitivity response to the antigen was apparent in each strain of mouse that was injected with 80 μg of HIS-62 mixed with Freund adjuvant. In addition, spleen cells from HIS-62-immunized mice proliferated in vitro in response to this antigen. Vaccination of each strain of mouse with 80 μg of HIS-62 conferred protection against a lethal intravenous challenge with *H. capsulatum* yeast cells. Thus, HIS-62 appears to be an important target of the cellular immune response to *H. capsulatum* and induces a protective immune response in mice.

Within mammalian tissues, the dimorphic fungal pathogen *Histoplasma capsulatum* survives as a yeast cell, and it is this form that is responsible for most if not all of the disease manifestations (5). The tissue phase of *H. capsulatum* binds to macrophages and is engulfed avidly by these phagocytes (2). Once inside this permissive milieu, yeast cells multiply; subsequently, the macrophages are stimulated by soluble products of T cells to inhibit replication (16, 22). Thus, an intact cell-mediated immune response that involves a collaborative interaction between T cells and macrophages is critical for a successful resolution of infection with this pathogenic fungus.

Since yeast cells are the parasitic form of the fungus, it is likely that, in tissues, T cells recognize an antigen derived from this phase. Although little information exists concerning the identity of antigens from yeast cells recognized by T cells, we have reported recently that a detergent extract from the cell wall and cell membrane (CW/M) of *H. capsulatum* yeast cells contained antigens recognized by T cells and protected C57BL/6 mice against a lethal challenge with *H. capsulatum* yeast cells (10). Moreover, the molecular weights of the antigens in this complex pool were determined by using T-cell immunoblotting analysis. A region of CW/M that encompassed 53 to 64 kDa stimulated proliferation of a T-cell line and several T-cell clones obtained from C57BL/6 mice (10).

In this study, we isolated a 62-kDa band, termed HIS-62, from electrophoresed CW/M of *H. capsulatum* yeast cells and examined its antigenicity and immunogenicity. The results indicate that this antigen is a target of the cellular immune response to *H. capsulatum* and confers protective immunity in mice against a lethal challenge with yeast cells.

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MATERIALS AND METHODS

Mice. C57BL/6 (*H-2*^{b}), BALB/c (*H-2*^{d}), and CBA/J (*H-2*^{k}) mice were purchased from Jackson Laboratory, Bar Harbor, Maine.

Injection of mice with *H. capsulatum* yeast cells. Preparation of *H. capsulatum* yeast cells was performed as described previously (10). To immunize mice with viable yeast cells, animals were subjected to the following series of injections. On day 0, mice received 10^6 yeast cells subcutaneously; on day 14, they were given an intravenous injection of 6×10^5 yeast cells; and on day 35, they were inoculated with 5×10^6 yeast cells intraperitoneally. In experiments employing a lethal challenge, mice were injected intravenously with 4×10^6 yeast cells.

Isolation of HIS-62. CW/M was prepared as described previously (10). Briefly, *H. capsulatum* yeast cells were cultured in Ham F-12 medium containing cysteine (8.4 μg/ml), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (6 g/liter), glutamic acid (1 g/liter), and glucose (18.2 g/liter) and were grown at 37°C in a shaking incubator for 48 h. Yeast cells were harvested and killed by incubation in phosphate-buffered saline (PBS) (pH 7.2), containing thimerosal (1:10,000, wt/vol) at room temperature for 1 h. Yeast cells were washed and resuspended in PBS containing 1 mM phenylmethylsulfonyl fluoride, 5 μM leupeptin, and 5×10^{-4} M disodium EDTA at a concentration of 1 volume of

packed cells to 2 volumes of buffer. Cells were disrupted in a bead beater (Biospec Products, Bartlesville, Okla.) at 4°C for 6 min. The homogenate was centrifuged at 450 × *g* for 5 min, and the supernatant and pellet were recovered. The supernatant was centrifuged at 11,000 × *g* for 20 min at 4°C and decanted. The pellets were pooled and washed three times with PBS. Particulate material was boiled in 125 mM Tris (pH 6.9) containing 6 M urea, 20 mM 2-mercaptoethanol, and 1% Tween 20 (vol/vol) and then incubated overnight at 4°C. The soluble material was separated by centrifugation at 11,000 × *g* for 20 min and dialyzed against PBS for 36 h. The protein content was 2.8 mg/ml.

To isolate HIS-62, CW/M was subjected to preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by using 3-mm 7.5% gels loaded with 6 to 12 mg of protein. After electrophoresis, the gels were stained for 20 min at room temperature with 0.3% Coomassie brilliant blue dissolved in 10% glacial acetic acid and 30% isopropyl alcohol (12). Gels were destained with a solution containing 5% glacial acetic acid and 16.5% methanol for 2 to 3 h at 4°C (12). The 62-kDa band was excised and soaked in water for 2 h. The section of gel was minced and placed in an electroelutor (Elutrap; Schleicher and Schuell, Keene, N.H.). Elution was performed at room temperature for 8 h at 200 V in a buffer containing 25 mM Tris, 192 mM glycine, and 0.1% SDS. The purity of the eluted fraction was checked by using SDS-PAGE minigels; if there were contaminating proteins, the material was electrophoresed and electroeluted a second time. SDS and Coomassie blue were extracted from the protein by the method of Konigsberg and Henderson (14). Antigen was suspended in PBS (pH 7.4) and filter sterilized before use. The protein content of HIS-62 was determined by the Bradford method (1).

SDS-PAGE of HIS-62. Three micrograms of antigen was electrophoresed in a 7.5% to 15% gradient polyacrylamide gel (15), and the gel was stained with silver (21).

In vivo injection of mice with HIS-62. The antigen was emulsified with an equal volume of complete Freund adjuvant or incomplete Freund adjuvant (Difco Laboratories, Detroit, Mich.). Initially, mice were injected subcutaneously with 40 μg of antigen in complete Freund adjuvant; 2 weeks later, they were injected with 40 μg of antigen in incomplete Freund adjuvant.

Induction and measurement of DTH response to HIS-62. Groups of six mice that were immunized with viable *H. capsulatum* yeast cells or with HIS-62 were challenged intradermally with 1 μg of antigen in a volume of 0.05 ml. Footpad swelling was measured 24 h later. The delayed-type hypersensitivity (DTH) response was expressed as the percent increase in footpad thickness from that measured immediately before antigen challenge (10). As a control, the DTH was measured in age-matched littermates that had been injected with an equal quantity of bovine serum albumin (BSA) in complete or incomplete Freund adjuvant. The data are expressed as the percent increase in footpad thickness. This value was calculated as [(thickness 24 h after injection of antigen − thickness before injection)/thickness before injection] × 100.

Organ culture for *H. capsulatum*. Spleens from mice infected with *H. capsulatum* were homogenized in PBS, and the homogenate was serially diluted. One hundred microliters of homogenate was dispensed onto plates containing brain heart infusion agar (2% [wt/vol] agar) supplemented with 5% (vol/vol) defibrinated sheep erythrocytes, 1% (wt/vol) dextrose, and 0.01% (wt/vol) cysteine hydrochloride. Plates were incubated at 30°C, and the CFU enumerated after 7 to 10 days.

Splenocyte preparation. Spleen cells from mice were prepared as described previously (10).

Generation of T-cell clones and T-cell hybridomas. A *H. capsulatum*-reactive murine T-cell line and T-cell clones that are $CD4^+$ from C57BL/6 mice were generated and propagated as described previously (6). $CD4^+$ T-cell hybridomas that recognize CW/M were isolated from BALB/c and CBA/J mice that had been immunized with viable *H. capsulatum* yeast cells. Spleen cells from immune BALB/c or CBA/J mice were suspended (3×10^6 cells per ml) in RPMI 1640 medium containing 10% fetal bovine serum and 10 μg of gentamicin per ml and stimulated with 5.6 μg of CW/M per ml for 4 days. Blast cells were harvested and fused with the thymoma BW5147.G.1.4 as described previously (4). Actively growing hybridomas that reacted to CW/M were expanded and maintained in Dulbecco modified essential medium containing 10% fetal bovine serum and 10 μg of gentamicin per ml. Hybridomas were cloned by limiting dilution at 0.3 cells per well in medium containing hypoxanthine, aminopterin, and thymidine. All clones were expanded and retested for reactivity to CW/M.

Splenocyte and T-cell stimulation assays. In the proliferation assay of splenocytes, 4×10^5 cells in 0.2 ml of RPMI 1640 containing 10% fetal bovine serum, 10 μg of gentamicin per ml, and 0.05 ml of antigen or medium were added to each well of a microtiter plate. For the proliferation assay of the murine T-cell line and cloned T cells, 2×10^4 resting cells in 0.1 ml of medium were incubated with 5×10^5 irradiated splenocytes suspended in 0.1 ml of medium and 0.05 ml of antigen or medium. Cultures of the T-cell line and T-cell clones were incubated for 72 h at 37°C in 5% CO_2, whereas cultures of splenocytes were incubated for 144 h; 16 h before cell harvest, 0.5 μCi of [^{3}H]thymidine (specific activity, 6.7 Ci/mmol; New England Nuclear, Boston, Mass.) was added to each well. T-cell hybridomas were suspended in RPMI 1640 supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% L-glutamine, 1% sodium pyruvate, 5×10^{-5} M 2-mercaptoethanol, and 10 μg of gentamicin per ml. To each well of a microtiter plate were added 3×10^4 T cells in 0.1 ml of medium, 3×10^4 LB 27.4 ($H\text{-}2^{b/d}$) cells or 3×10^4 LK 35.2 ($H\text{-}2^{k/d}$) cells, and 0.05 ml of antigen. LB cells were used as antigen-presenting cells for T-cell hybridomas derived from BALB/c mice, and LK cells were used as antigen-presenting cells for CBA/J-derived T-cell hybridomas. Cultures were incubated for 24 h at 37°C in 5% CO_2. Subsequently, 100 μl of supernatant were removed and tested for interleukin 2 activity.

In stimulation assays, the protein concentration of CW/M in a well was 5.6 μg/ml and that of HIS-62 was 5 μg/ml. In multiple preliminary experiments, these concentrations were found to be optimal. The responses by cells to the antigen were considered positive when they were greater than or equal to threefold higher than the response of cells incubated with medium alone.

Assay of interleukin 2 activity. Test supernatants were added to 4×10^3 CTLL-2 cells in 0.1 ml of medium and cultured at 37°C in 5% CO_2 for 28 h. At 4 h before harvest, cells were pulsed with 1 μCi of [^{3}H]thymidine.

Statistics. The Mantel-Haenszel test was used to analyze the survival of *H. capsulatum*-infected mice immunized with HIS-62 or with BSA (17). A P of <0.05 was considered significant.

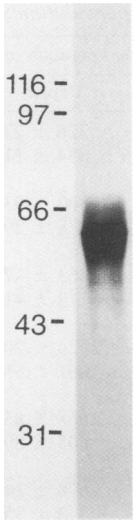

FIG. 1. SDS-PAGE analysis of HIS-62 with a 7.5 to 15% polyacrylamide gradient gel stained with silver. Molecular weight markers (10^3) are depicted on the left of the gel.

RESULTS

Electrophoretic appearance of HIS-62. A Coomassie blue-stained band corresponding to 62 kDa was excised from polyacrylamide gels of CW/M and electroeluted in Tris-glycine-SDS. The eluted protein appeared as a single band in a silver-stained gel (Fig. 1). All assays were performed with preparations of HIS-62 that had a single band as determined by staining the gels with Coomassie blue and silver.

Mice injected with viable *H. capsulatum* yeast cells or with HIS-62 mount a DTH response to this antigen. C57BL/6, BALB/c, and CBA/J mice were injected with viable *H. capsulatum* yeast cells or with 80 μg of HIS-62 in Freund adjuvant, and then the footpads were tested with 1 μg of HIS-62 2 weeks after the last immunization. Mice immunized with yeast cells mounted a significant DTH response to HIS-62 as compared with that of unimmunized controls (Table 1). In addition, the DTH responses by mice immunized with HIS-62 significantly exceeded those of mice injected with BSA in Freund adjuvant (Table 1).

Splenocytes from mice injected with yeast cells or with HIS-62 proliferate in vitro to HIS-62. Splenocytes from individual C57BL/6, BALB/c, and CBA/J mice that had been immunized with viable *H. capsulatum* yeast cells or from controls were tested for their capacity to recognize HIS-62. For each strain, splenocytes from six individual mice were tested. The results from three mice of each strain are shown in Table 2. Spleen cells from all immunized mice proliferated in response to the antigen, whereas splenocytes from unimmunized animals did not.

Similarly, splenocytes from six individual C57BL/6, BALB/c, and CBA/J mice that had been immunized with 80 μg of HIS-62 recognized this antigen. In contrast, spleen cells from all mice given BSA did not respond to HIS-62. The data for three mice of each strain are presented in Table 3.

Recognition of HIS-62 by monoclonal populations of T cells. We reported previously that T-cell immunoblot analysis indicated that a region of nitrocellulose-bound CW/M encompassing 62 kDa stimulated a murine T-cell line and T-cell clones derived from C57BL/6 mice (10). Therefore, studies were performed to determine whether the line and T-cell clones could respond to HIS-62. The T-cell line JC1 and all five clones responded to the antigen (Table 4).

In the next series of experiments, T-cell hybridomas isolated from BALB/c and CBA/J mice that had been immunized with *H. capsulatum* were tested for their capacity to respond to HIS-62 or, as a control, CW/M. Ten of 15 BALB/c T-cell hybridomas and 8 of 12 CBA/J hybridomas produced interleukin 2 in response to HIS-62 (Tables 5 and 6). In some cases (e.g., B10II3A3 and B60B4), the response to HIS-62 was modest as compared with the response to CW/M. Moreover, the hybridomas that did not recognize HIS-62 did produce interleukin 2 in response to CW/M. Taken together, the data indicate that HIS-62 is an immunodominant antigen that is recognized by a high proportion of monoclonal populations of T cells from each of the three strains of mice.

Protective efficacy of HIS-62. Previously, we had demonstrated that injection of CW/M in Freund adjuvant could mediate protection in C57BL/6 mice against a lethal intravenous challenge with *H. capsulatum* yeast cells (10). Since HIS-62 was a target of the cellular immune response to *H. capsulatum*, we sought to determine whether this antigen could confer protective immunity in C57BL/6, BALB/c, and CBA/J mice. Groups of six mice were injected in two divided doses with a total of 80 μg of HIS-62 in Freund adjuvant; as a control, separate groups of mice were injected with 80 μg of BSA in Freund adjuvant. Two weeks after the last injection, mice were challenged intravenously with 4×10^6 yeasts and then observed for 28 days.

All infected mice injected with BSA died by day 11 of infection (Fig. 2). In contrast, five of six HIS-62-immunized mice from each strain survived for 28 days (Fig. 2). Of the HIS-62-immunized mice that died, a C57BL/6 mouse died on day 7 of infection, a BALB/c mouse died on day 18, and a

TABLE 1. DTH response to HIS-62 by mice immunized with *H. capsulatum* yeast cells or HIS-62

Strain	Immunization[a]	% Increase in footpad thickness (mean ± SEM)[b]
C57BL/6	None	3 ± 2
	H. capsulatum yeast cells	24 ± 5[c]
	BSA (80 μg)	3 ± 1
	HIS-62 (80 μg)	16 ± 2[c]
BALB/c	None	2 ± 1
	H. capsulatum yeast cells	32 ± 3[c]
	BSA (80 μg)	1 ± 1
	HIS-62 (80 μg)	13 ± 2[c]
CBA/J	None	4 ± 2
	H. capsulatum yeast cells	37 ± 4[c]
	BSA (80 μg)	3 ± 1
	HIS-62 (80 μg)	19 ± 2[c]

[a] Mice were injected with viable *H. capsulatum* yeast cells as described in Materials and Methods. One half of the total dose of BSA or HIS-62 was injected subcutaneously in complete Freund adjuvant; after 2 weeks, the other half of the dose was given in incomplete Freund adjuvant. Mice were tested 2 weeks later.

[b] Results for groups of six mice tested with 1 μg of HIS-62.

[c] Differences between footpad thicknesses of control and immunized mice were significant ($P < 0.01$).

TABLE 2. Recognition of HIS-62 by splenocytes from mice immunized with *H. capsulatum* yeast cells

Strain	Immunization	Stimulus	[^{3}H]thymidine incorporation (mean cpm ± SEM)[a]		
			Expt 1	Expt 2	Expt 3
C57BL/6	None	Medium	274 ± 124	726 ± 272	244 ± 16
		HIS-62[b]	166 ± 58	834 ± 219	186 ± 29
	H. capsulatum yeast cells	Medium	725 ± 39	300 ± 93	739 ± 162
		HIS-62[b]	11,470 ± 394	6,678 ± 29	7,284 ± 377
BALB/c	None	Medium	3,916 ± 753	6,463 ± 474	2,553 ± 251
		HIS-62[b]	4,128 ± 201	4,112 ± 416	2,973 ± 353
	H. capsulatum yeast cells	Medium	2,662 ± 1,281	2,324 ± 208	2,584 ± 195
		HIS-62[b]	39,291 ± 2,480	44,061 ± 681	27,838 ± 1,106
CBA/J	None	Medium	5,063 ± 1,164	2,668 ± 831	2,959 ± 121
		HIS-62[b]	3,487 ± 341	805 ± 89	3,588 ± 1,413
	H. capsulatum yeast cells	Medium	458 ± 12	678 ± 147	2,284 ± 500
		HIS-62[b]	4,310 ± 41	6,848 ± 35	51,176 ± 5,097

[a] Results from triplicate determinations.
[b] Final concentration of HIS-62, 5 μg/ml.

CBA/J mouse died on day 16. When analyzed statistically, the survival of antigen-immunized animals was significantly (C57BL/6, $P = 0.024$; BALB/c, $P = 0.004$; CBA/J, $P = 0.003$) different from that of controls injected with BSA. Sampling of spleens from BSA-injected mice that had died revealed the presence of $>10^7$ CFU of *H. capsulatum*; no bacteria were detected. Cultures of spleens from HIS-62-immunized animals that survived the lethal challenge revealed the presence of comparatively few *H. capsulatum* CFU per spleen. For 14 of 15 survivors, the number of CFU ranged from <100 to 2,000. A spleen from one survivor contained 46,750 CFU. Thus, during the 28-day observation period, HIS-62 mediates a protective immune response.

DISCUSSION

The host response to *H. capsulatum* is directed principally, if not exclusively, to yeast-phase organisms, since those are the forms that induce disease and are responsible for the pathogenesis of histoplasmosis (5). Therefore, a series of studies has been undertaken by our laboratory to identify and to isolate antigens from *H. capsulatum* yeast cells recognized by T cells. Previous work demonstrated that a detergent extract of the cell wall and cell membrane from yeast cells contained antigens that stimulated *H. capsulatum*-reactive T-cell clones from C57BL/6 mice. T-cell immunoblot analysis revealed that a region spanning 53 to 64 kDa contained an antigen or antigens recognized by a majority of the T-cell clones (10). Furthermore, the extract confers protective immunity in C57BL/6 mice (10).

In this study, we have explored the possibility that a protein present in the 53- to 64-kDa region of CW/M was antigenic or immunogenic or both not only for C57BL/6 mice but also for two additional strains of inbred mice, BALB/c and CBA/J, that differ genetically from C57BL/6. This protein was selected because it constituted the most prominent band in that region of the polyacrylamide gels. Furthermore, initial studies of nitrocellulose-bound CW/M that had been electrophoresed revealed that the 62-kDa band was antigenic (9).

The results indicated that C57BL/6, BALB/c, and CBA/J mice immunized with viable *H. capsulatum* yeast cells

TABLE 3. Proliferative response to HIS-62 by splenocytes from HIS-62 immunized mice

Strain	Immunization[a]	Stimulus	[^{3}H]thymidine incorporation (mean cpm ± SEM)[b]		
			Expt 1	Expt 2	Expt 3
C57BL/6	BSA	Medium	1,291 ± 248	512 ± 201	622 ± 98
		HIS-62[c]	406 ± 125	584 ± 213	601 ± 66
	HIS-62	Medium	200 ± 80	1,326 ± 183	701 ± 221
		HIS-62[c]	9,030 ± 92	35,786 ± 2,878	14,989 ± 148
BALB/c	BSA	Medium	3,561 ± 659	7,596 ± 1,003	4,938 ± 1,732
		HIS-62[c]	2,360 ± 254	9,510 ± 1,199	2,868 ± 236
	HIS-62	Medium	2,061 ± 351	1,640 ± 308	1,505 ± 253
		HIS-62[c]	30,084 ± 2,469	22,954 ± 862	17,042 ± 1,172
CBA/J	BSA	Medium	797 ± 201	2,925 ± 996	1,338 ± 590
		HIS-62[c]	758 ± 148	735 ± 87	1,571 ± 308
	HIS-62	Medium	495 ± 127	1,778 ± 150	1,869 ± 464
		HIS-62[c]	11,472 ± 236	45,747 ± 3,350	24,736 ± 733

[a] BSA and HIS-62 were injected at doses of 80 μg per mouse.
[b] Results from triplicate determinations.
[c] Final concentration of HIS-62, 5 μg/ml.

TABLE 4. Proliferative response to HIS-62 by a T-cell line and T-cell clones from C57BL/6 mice

Line or clone	[^{3}H]thymidine incorporation (cpm ± SEM)[a]	
	Medium	HIS-62[b]
JC1	1,374 ± 293	14,912 ± 1,123
2.3H3	704 ± 30	10,051 ± 533
1.3G6	864 ± 12	9,604 ± 1,572
1.1E1	399 ± 56	3,382 ± 240
1.1C3	602 ± 94	22,804 ± 1,658
1.3D1	368 ± 39	9,289 ± 272

[a] Results from triplicate determinations (one representative experiment of at least two).
[b] Final concentration of HIS-62, 5 μg/ml.

mounted a DTH response to HIS-62 and that splenocytes from these animals responded to this antigen in vitro. Inoculation of each strain of mouse with this antigen in Freund adjuvant induced cutaneous DTH reactivity and in vitro blastogenic responses by spleen cells to this antigen. Additional experiments demonstrated that HIS-62 stimulated a high proportion (67 to 100%) of monoclonal populations of T cells derived from each strain. This antigen also conferred a significant protective immune response in each mouse strain. Thus, this antigen not only activates T cells but also stimulates a protective immune response.

We have focused our analysis of this antigen on the cellular immune responses to it because there is little evidence that humoral immunity is important in host resistance to *H. capsulatum*. Transfer of sera containing anti-*Histoplasma* antibodies to naive mice fails to modify the severity of infection with *H. capsulatum* yeast cells (13). In addition, anti-*Histoplasma* antibody is not required for ingestion by phagocytes, nor is it requisite for opsonization, since human serum devoid of anti-*Histoplasma* antibody efficiently opsonizes *H. capsulatum* yeast cells (2, 19).

The biochemical nature of HIS-62 has not been determined in this study, since the intent was to determine the functional importance of this antigen in T cell-mediated

TABLE 5. Response by T-cell hybridomas from BALB/c mice to HIS-62

Hybridoma	[^{3}H]thymidine incorporation by CTLL-2 cells (mean ± SEM)[a]		
	Medium	HIS-62[b]	CW/M[c]
B60A5	396 ± 15	18,162 ± 928	9,587 ± 146
B4IID1	661 ± 67	32,027 ± 2,382	33,188 ± 2,890
B4IIC5	423 ± 61	9,617 ± 883	12,567 ± 468
B4IIA6	443 ± 43	32,116 ± 1,866	32,547 ± 846
B4IIB6	537 ± 64	24,963 ± 855	25,417 ± 1,045
B60C1	239 ± 102	13,820 ± 1,277	7,286 ± 944
B4IIB1	451 ± 44	29,211 ± 380	12,042 ± 285
B10II3A3	457 ± 41	4,186 ± 359	16,644 ± 661
B60B4	662 ± 120	7,442 ± 1,217	35,872 ± 1,322
B10II3B6	451 ± 44	3,328 ± 40	14,961 ± 669
B60IIIC1	392 ± 86	1,091 ± 136	10,586 ± 636
B10II3C2	517 ± 48	1,210 ± 195	30,818 ± 833
B60IIIA6	590 ± 53	625 ± 103	11,238 ± 1,098
B10II3C3	190 ± 11	356 ± 87	6,581 ± 298
B60IIIB5	385 ± 48	665 ± 114	5,741 ± 358

[a] Results from triplicate determinations (one representative experiment of two).
[b] Final concentration of HIS-62, 5 μg/ml.
[c] Final concentration of CW/M, 5.6 μg/ml.

TABLE 6. Response by T-cell hybridomas from CBA/J mice to HIS-62

Hybridoma	[^{3}H]thymidine incorporation by CTLL-2 cells (mean ± SEM)[a]		
	Medium	HIS-62[b]	CW/M[c]
C23C3.30B1	209 ± 32	9,331 ± 120	6,949 ± 162
C23C3.30C6	258 ± 19	11,023 ± 717	8,976 ± 810
C25C2.A4	197 ± 44	5,750 ± 379	4,900 ± 249
C15A4.A4	97 ± 9	6,179 ± 396	4,591 ± 136
C15A4.A2	67 ± 11	8,812 ± 233	6,205 ± 141
C23C3.30B6	114 ± 8	5,383 ± 658	4,529 ± 145
C15A4.C1	236 ± 71	10,240 ± 440	6,939 ± 382
C15A4.C3	143 ± 36	8,270 ± 434	4,824 ± 75
C23C3.30D1	396 ± 36	661 ± 45	9,202 ± 578
C1B4.A1	737 ± 102	1,378 ± 101	20,638 ± 1,184
C9A2.A1	2,747 ± 42	2,280 ± 87	10,784 ± 565
C1B4.A4	592 ± 57	1,505 ± 178	23,684 ± 89

[a] Results from triplicate determinations (one representative experiment of two).
[b] Final concentration of HIS-62, 5 μg/ml.
[c] Final concentration of CW/M, 5.6 μg/ml.

responses. On silver-stained gels, the band is somewhat diffuse, which suggests that this is a glycoprotein. Preliminary evidence from our laboratory indicates that HIS-62 is a glycoprotein, since it bound concanavalin A and wheat germ agglutinin during lectin blot analysis (11). Further work is in progress to analyze the biochemical nature of this antigen, including its amino acid sequence and carbohydrate composition.

Information concerning the identity and isolation of immunogens from *H. capsulatum* yeast cells is limited. Initially, it was shown that injection of viable or heat-killed yeast cells could mediate protective immunity (18). Then several reports provided evidence that extracts from *H. capsulatum* yeast cells contained protective molecules. Garcia and Howard (7) demonstrated that an ethylenediamine extract of intact yeast cells was protective. Others have described a ribosome-protein complex from *H. capsulatum* yeast cells that induces protective immunity in mice (20). In addition, CW/M, a detergent extract of yeast cells, conferred protection in C57BL/6 mice against a lethal challenge of *H. capsulatum* yeast cells (10). The current study clearly demonstrated that an antigen from CW/M, rather than a complex pool of antigens, was protective against a lethal challenge in BALB/c and CBA/J mice as well as C57BL/6 mice. To our knowledge this is the first report of a more highly purified antigen from *H. capsulatum* yeast cells that can stimulate a protective immune response.

The mechanism or mechanisms by which HIS-62 stimu-

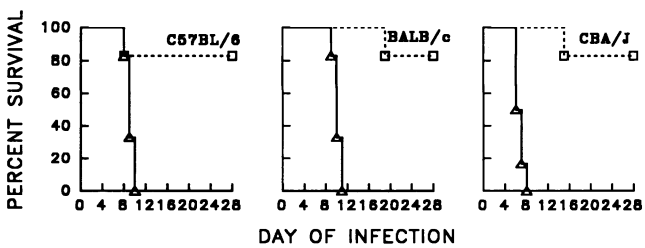

FIG. 2. Survival profiles of C57BL/6, BALB/c, and CBA/J mice immunized with 80 μg of HIS-62 (□) or 80 μg of BSA (△). All mice were challenged with 4×10^6 *H. capsulatum* yeast cells.

lates host resistance to *H. capsulatum* have not been defined completely. The data strongly suggest that T cell-mediated responses are involved, since this antigen does induce cellular immune responses in mice. On the other hand, it is unlikely that humoral immune responses contribute for the reasons discussed above. One mechanism that may be engaged is the activation of $CD4^+$ T cells. Elimination of these cells from normal mice increases susceptibility to a challenge with *H. capsulatum* (8). Also, $CD4^+$ but not $CD8^+$ T cells from spleens of mice immunized with viable yeast cells can transfer protective immunity (3). Thus, HIS-62 may activate $CD4^+$ T cells that, in turn, arm macrophages to limit infection with *H. capsulatum*.

One important consideration in the investigation of protective antigens is whether responses to the immunogen are restricted genetically. Thus, in the present report, three common strains of inbred mice that differ genetically were examined for their ability to recognize HIS-62. In each strain, the antigen induced cellular immune responses and protective immunity. In this limited analysis, there was no evidence of genetic restriction. Nevertheless, examination of many other strains will be necessary before definite conclusions can be drawn about the genetic influences on responses to HIS-62. The fact that a high proportion of the antigen-reactive monoclonal T cells responded to HIS-62 suggests that it is an immunodominant antigen. However, it also should be emphasized that approximately 33% of the T cells from BALB/c and CBA/J mice did not respond to this antigen. Therefore, other antigens are present in CW/M that stimulate T cells from these two strains of mice. The identity of these antigens and the role they may play in host resistance remain to be determined.

In summary, we have isolated a 62-kDa antigen from a detergent extract of the cell wall and cell membrane of *H. capsulatum* yeast cells. This antigen, HIS-62, is an important target of the cellular immune response to this pathogenic fungus. Furthermore, it induces T cell-mediated responses and protective immunity. Studies are underway to determine which peptides from this antigen possess the capacity to stimulate T cells and mediate protection.

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