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Production of Antibodies to Antigens of *Candida albicans* in CBA/H Mice

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Reported targets of the specific immune responses to *Candida albicans* in human candidiasis include a 47-kDa breakdown product of a 90-kDa heat shock protein (HSP 90) (R. Matthews and J. Burnie, FEMS Microbiol. Lett. 60:25–30, 1989) and the 48-kDa enolase (K. M. Franklyn, J. R. Warmington, A. K. Ott, and R. B. Ashman, Immunol. Cell Biol. 68:173–178, 1990). These proteins are immunodominant antigens of *C. albicans*. Western blotting (immunoblotting) and immunoprecipitation were used to investigate the humoral response in a mouse model of systemic candidiasis. Resolution of systemic candidiasis in CBA/H mice is associated with a high level of antibody reactivity to *C. albicans* antigens. A significant antibody response against a non-HSP antigen of 96 kDa which was distinct from the *C. albicans* HSP 90 antigen was detected. Significant antibody reactivity against an HSP of 75 kDa was also detected. We concluded that resolution of *C. albicans* infections in CBA/H mice was associated with antibodies to an HSP and a non-HSP of 75 and 96 kDa, respectively.

Host defense mechanisms against infection with *Candida albicans* are complex. Many studies have demonstrated essential roles for phagocytic cells, classical cell-mediated immunity, and humoral immune responses in the resolution of *C. albicans* infections (16). Deficiencies in any system have been linked to increased susceptibility to *C. albicans* in the host (reviewed by Greenfield [7]).

In humans, there is considerable heterogeneity of the humoral responses to antigens of *C. albicans* (12, 17). Several immunodominant antigens of *C. albicans* have been identified. These include a 47-kDa breakdown product of the 90-kDa heat shock protein (HSP 90) (11) and a 48-kDa antigen (enolase) (6). Matthews et al. (13) have shown that patients recovering from systemic candidiasis produce antibodies to the 47-kDa antigen of *C. albicans*.

Analysis of the antibody responses during systemic candidiasis in inbred mice permits the characterization of specific responses in a species of limited genetic diversity. CBA/H mice exhibit a transient susceptibility to systemic infection with *C. albicans* (1, 2, 8). The CBA/H mice show substantial tissue colonization 3 to 4 days postinfection with a sublethal dose of *C. albicans*, particularly in the brain and kidney tissues. However, the infection is resolved by day 10. High titers of antibodies in sera from CBA/H mice recovering from primary *C. albicans* infection suggest that antibodies may play a role in the resolution of the infection (8).

We have analyzed the humoral response of the CBA/H mice to systemic infection with *C. albicans*. Antibodies of several specificities were detected. The major antibody response identified was directed against a protein antigen of 96 kDa. This protein was different from the previously described HSP 90 of *C. albicans* (11, 14). The antibody reactivity was also directed against an HSP of 75 kDa.

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MATERIALS AND METHODS

Mice. Six- to 8-week-old female CBA/H mice were purchased from the Animal Resource Centre, Perth, Western Australia, Australia. Techniques used for bleeding and injection of mice were approved by the Animal Experimentation Ethics Committee of Curtin University of Technology.

Culture methods. A clinical isolate of *C. albicans* (KEMH 5) was obtained from the Department of Pathology, King Edward Memorial Hospital, Subiaco, Perth, Western Australia, Australia. The organism was grown in yeast extract-peptone-dextrose broth (1% yeast extract, 2% peptone, 2% glucose) overnight at 30°C in a shaking water bath.

Temperature stressing and radiolabelling of cells. An overnight culture of *C. albicans* was pelleted by centrifugation and washed twice with 0.9% sterile saline. The cell pellet was resuspended in yeast nitrogen base containing 1% (wt/vol) glucose (supplemented with 0.1 mmol of tryptophan per liter) and incubated at 30°C for 5 to 8 h or until the culture reached early- to mid-log-phase growth. An aliquot (100 ml) of mid-log-phase *C. albicans* cells was transferred to 100 ml of fresh yeast nitrogen base at 42°C and incubated for 30 min with 100 μCi of ^{35}S-translabelled methionine and cysteine (70% L-methionine and 15% cysteine; ICN Biomedicals Inc.). The remaining mid-log-phase culture was incubated for a further 30 min at 30°C with 100 μCi of trans ^{35}S label. After 30 min, the labelling reaction was terminated by the addition of 500 μl of cold chase mixture containing unlabelled amino acids (65 μmol of methionine and 65 μmol of cysteine per liter). The sample was pelleted by centrifugation at 2,000 × *g* for 10 min at 4°C and washed twice in 0.9% sterile saline at 4°C.

***C. albicans* whole yeast extract.** Heat-stressed cells of *C. albicans* (10^9 blastoconidia) were centrifuged and washed as described above. The cell pellet was resuspended in 10 ml of protein extraction buffer (100 mmol of Tris-HCl [pH 8.0], 14 mmol of β-mercaptoethanol, 1 mmol of EDTA [pH 8.0], and 1 mmol of phenylmethylsulfonyl fluoride per liter). An equal volume of 0.5-mm-diameter glass beads, pretreated by acid washing and Repelcote (BDH), was added to the cell suspen-

sion. The cells were vortexed for 1 min and cooled on ice for 1 min. This cycle was repeated until at least 80% of the cells were lysed or spheroplasted as determined by phase-contrast microscopic examination. Sodium dodecyl sulfate (SDS) was added to a final concentration of 1%. The glass beads were separated from the yeast antigen preparation by punching a hole smaller than 0.5 mm in the bottom of the tube and centrifuging the supernatant into a clean tube. The extracted yeast antigen preparations were clarified by centrifugation and stored at −80°C until required.

Generation of immune serum. Ten CBA/H mice were inoculated intravenously via the tail vein with 3×10^5 washed blastoconidia of *C. albicans*. Pooled sera were obtained on days 10 and 24 from infected mice. The sera were stored at −20°C.

SDS-PAGE and Western blotting (immunoblotting). Protein samples were denatured and reduced by heating for 5 min at 100°C in 74 mmol of Tris-HCl (pH 6.8) per liter containing 843 mmol of β-mercaptoethanol per liter, 11.7% (vol/vol) glycerol, 2.4% (wt/vol) SDS, and 0.05% (wt/vol) bromophenol blue. The solubilized antigen preparations were electrophoresed on sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis (SDS-PAGE) gels or on 7.5 to 12.5% prepoured SDS-PAGE gels (Bio-Rad). After electrophoresis, the proteins were electroblotted onto a nitrocellulose membrane (Bio-Rad). A molecular mass standard (Bio-Rad) containing lysozyme (14.4 kDa), soybean trypsin inhibitor (21.5 kDa), carbonic anhydrase (31 kDa), ovalbumin (45 kDa), bovine serum albumin (66.2 kDa), and phosphorylase *b* (97.4 kDa) was electrophoresed in parallel with the test samples. The molecular masses of the *C. albicans* proteins were determined by scanning an image of the electrophoresed gradient gel into the National Centre for Supercomputing Applications GelReader v1.0.1 program on a Macintosh IIcx computer.

Antibody detection. *Candida*-specific antibodies in the sera of infected mice were detected by immunostaining as described by Mierendorf et al. (15), with the following modifications. Nonspecific binding of antibody solutions to nitrocellulose was prevented by blocking free protein-binding sites with 1% bovine serum albumin in phosphate-buffered saline containing 0.05% Tween 20 (PBS-Tw) (pH 7.4). The nitrocellulose blots were incubated with immune sera (primary antibody; 1/50 final dilution in PBS-Tw) at 25°C for 90 min with gentle agitation. The nitrocellulose blots were washed three times in excess PBS-Tw at 25°C for 5 min and incubated with sheep anti-mouse immunoglobulin G (IgG) alkaline phosphatase-conjugated antibody (secondary antibody; Silenus Laboratories Pty Ltd., Victoria, Australia). The secondary antibody was routinely preadsorbed with *C. albicans* whole yeast extract (previously heat treated at 100°C for 5 min) for 2 h at 25°C. The secondary antibody solutions were prepared in PBS-Tw as 1/2,000 final dilutions and incubated with the nitrocellulose blots at 25°C for 60 min. After three 5-min washes in PBS, the antigen-antibody complexes were detected by monitoring the deposition of purple stain following reaction with nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate in dimethylformamide. The substrates were prediluted in a mixture of 100 mmol of Tris-HCl, 100 mmol of NaCl, and 50 mmol of $MgCl_2$ per liter.

Immunoprecipitation. A modified immunoprecipitation procedure based on the methods described by Zhong and Brunham (21) and Ey et al. (5) was used. Unless otherwise stated, all procedures were performed at room temperature. A 10-μl aliquot of test antigen (10 mg/ml) was mixed with 40 μl of immune serum in a microcentrifuge tube (Beckman), and 20 μl of washing buffer containing 50 mmol of Tris-HCl (pH 7.4) per liter and 0.15 M NaCl was added to each tube. The antigen preparations used were from heat-stressed or unstressed cells of *C. albicans*. The tubes were incubated for 1 h with gentle agitation. Protein A–Sepharose CL-4B (Pharmacia, Uppsala, Sweden) was allowed to swell in washing buffer for 1 h. Prior to use, contaminating protein and loosely bound protein A were removed by washing the Sepharose twice with excess 0.1 M sodium citrate (pH 3.3) containing 0.05% sodium azide at 4°C. The protein A-Sepharose was reequilibrated by rinsing with washing buffer. A 40-μl volume of a 30-mg/ml suspension of protein A-Sepharose was added to each reaction mixture and further incubated for 1 h with gentle agitation. The tubes were centrifuged briefly, and the supernatant was discarded. The protein A-Sepharose pellets were washed five times with 1 ml of wash buffer, resuspended in SDS reducing buffer, and heated at 100°C for 10 min. The reduced samples were fractionated on an SDS–10% PAGE gel. Proteins on the gel were visualized by staining with Coomassie blue. Proteins on a duplicate gel were electroblotted onto nitrocellulose and immunostained with immune mouse sera. Radiolabelled proteins on the stained gel or the immunostained blot were visualized after exposure to Kodak X-OMAT AR film at −80°C for 48 h with an intensifying screen (Dupont).

RESULTS

Antibodies to antigens of *C. albicans*. An extract of whole cells of *C. albicans* was subjected to gradient SDS-PAGE and electroblotted onto nitrocellulose. The immunoreactivity of immune sera from CBA/H mice injected 10 days previously with a sublethal dose of 3×10^5 blastoconidia of *C. albicans* was analyzed by immunostaining. Tissue colonization levels in both the brain and kidney peaked at day 4 postinfection (16a). The mean CFU per gram of tissue at day 4 postinfection was 2×10^6 in the kidney and 2.4×10^4 in brain tissue. A dose of 10^6 *C. albicans* blastoconidia per mouse was found to be lethal (dose-response data not shown). Typically, immunoreactivity to a total of 28 bands could be visualized by this method (Fig. 1). Serum taken from CBA/H mice prior to inoculation and analyzed by immunostaining showed no immunological reactivity to *C. albicans* antigens (Fig. 1, lane 1). Six bands of 46, 48, 79, 87, 96, and 138 kDa were more intensely stained than the others with immune sera (Fig. 1, lanes 2 and 3). These antigens were considered immunodominant with respect to the humoral immune response of CBA/H mice to *C. albicans*. The predominant reactivity, shown by the highest intensity of staining, was directed towards an antigen of 96 kDa (Fig. 1). HSPs, including those of the 90-kDa class, are major targets for the immune system in many infections (14, 18, 19). Further studies were performed to determine whether any of the immunodominant antigens of *C. albicans* were HSPs.

Immunoreactivity to HSPs. Extracts from unstressed or heat-stressed cells of *C. albicans* were separated by gradient SDS-PAGE and electroblotted onto a nitrocellulose membrane. Immune sera from CBA/H mice (day 24 postinfection) were analyzed by immunostaining for reactivity against the antigens of unstressed or heat-stressed *C. albicans* cell extracts. A second, duplicate SDS-PAGE gel was stained with Coomassie blue, dried, exposed to autoradiographic film, and used for molecular mass determination (data not shown). Antigens from both the unstressed and stressed cells were reactive with immune sera from CBA/H mice (Fig. 2). Immunoreactivity to the immunodominant 96-kDa antigen was demonstrated in extracts from both stressed and unstressed *C. albicans* cells (Fig. 2). The relative intensity of staining of the 96-kDa antigen in the sample from unstressed cells of *C. albicans* was greater than that of the antigens from heat-stressed cells (Fig. 2;

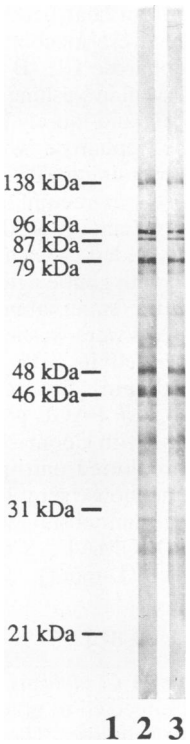

FIG. 1. Immunoblot of antigen extracts from *C. albicans* that have been fractionated by SDS-PAGE. The immunoblot was screened with day zero preimmune (control) sera and day 10 CBA/H immune sera and probed with alkaline phosphatase-conjugated anti-mouse immunoglobulin (gamma and light chain specific). Lanes: 1, *C. albicans* antigen extract reacted with preimmune sera; 2 and 3, duplicate samples of *C. albicans* antigen extract reacted with day 10 immune sera.

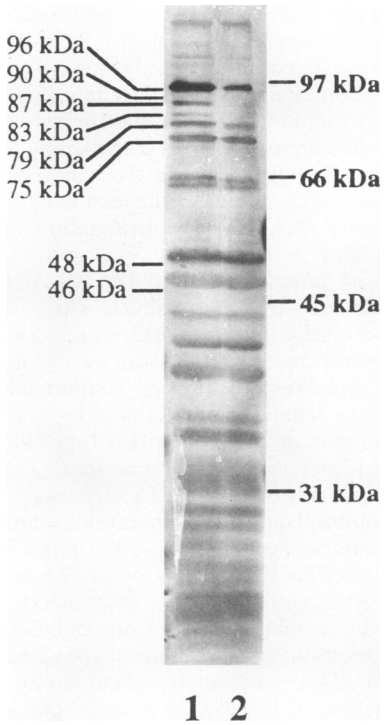

FIG. 2. Immunoblot of antigen extracts of heat-stressed and unstressed *C. albicans* that have been fractionated by SDS-PAGE. The immunoblot was screened with day 24 CBA/H immune sera and probed with alkaline phosphatase-conjugated anti-mouse immunoglobulin (gamma and light chain specific). Lanes: 1, antigen extract from unstressed *C. albicans*; 2, antigen extract from heat-stressed *C. albicans.*

compare lane 1 with lane 2). Staining of the 83- and 87-kDa antigens was not detected in the extract from heat-stressed *C. albicans* (Fig. 2, lane 2). Additionally, strong immunoreactivity to a 75-kDa antigen of *C. albicans* was detected in the antigen extracts from stressed and unstressed cells (Fig. 2).

The heat shock profile of separated proteins was determined by directly exposing the immunostained nitrocellulose blot to autoradiographic film for 3 days at −70°C with an intensifying screen (Fig. 3). The radioactivities of the different proteins from the extract of unstressed *C. albicans* showed the normal constitutive level of protein expression (Fig. 3, lane 1). Increased expression of the HSPs was demonstrated by more intense labelling of the bands in the extract from heat-stressed *C. albicans* cells (Fig. 3; compare lane 1 with lane 2, especially in the 70- to 90-kDa region). In addition, the extract from the heat-stressed cells exhibited bands not detected in the extract from unstressed *C. albicans*. The 96-kDa antigen was not radiolabelled in the extract from heat-stressed cells (Fig. 3, lane 2). Therefore, the immunodominant 96-kDa antigen was identified as a non-HSP.

A 90-kDa protein was strongly radiolabelled in the antigen extract from heat-stressed *C. albicans* (Fig. 3, lane 2). Expression of the 90-kDa protein was weak in the sample from unstressed cells (Fig. 3; compare lane 1 with lane 2). The major 90-kDa HSP was not immunoreactive with immune CBA/H sera (compare Fig. 2, lane 2, with Fig. 3, lane 2). A 75-kDa protein was radiolabelled in the antigen extract from heat-stressed cells and was identified as an HSP (Fig. 3, lane 2). The 75-kDa HSP was immunoreactive with immune CBA/H sera (Fig. 2).

Immunoprecipitation. Sera from immunized CBA/H mice were evaluated by immunoprecipitation for IgG class antibodies binding with antigens from a cell extract of the radiolabelled *C. albicans* culture. The cell extract was fractionated by SDS-PAGE. The proteins were transferred by electroblotting to a nitrocellulose membrane and immunoreacted with immune sera from CBA/H mice. The relative molecular weights of the proteins were estimated by comparison with standards on a duplicate SDS-PAGE gel stained with Coomassie blue (not shown).

Immunoprecipitated proteins were identified by comparing the immunoreactivity of the immunoprecipitation samples (Fig. 4, lanes 3 and 4) with that of the proteins from the heat-stressed and unstressed *C. albicans* cell extracts (Fig. 4, lanes 1 and 2). The 96-kDa antigen was the predominant immunoprecipitated antigen and was precipitated from both the stressed and unstressed *C. albicans* antigen preparations (Fig. 4, lanes 3 and 4, position a). A fainter band at 75 kDa was also immunoprecipitated (Fig. 4, lane 3). The immunoprecipitated 96-kDa antigen was not radiolabelled (Fig. 5, lane 3, position a). The predominant HSP in the extract from heat-stressed *C. albicans* was located at 90 kDa (Fig. 5, lane 1). A radiolabelled band was not detected in the immunoprecipitated antigen extract from stressed *C. albicans* at the corresponding 90-kDa position (Fig. 5, lane 3). A single radiolabelled protein of 75 kDa was present in the immunoprecipitated extract from heat-stressed *C. albicans* (Fig. 5, lane 3).

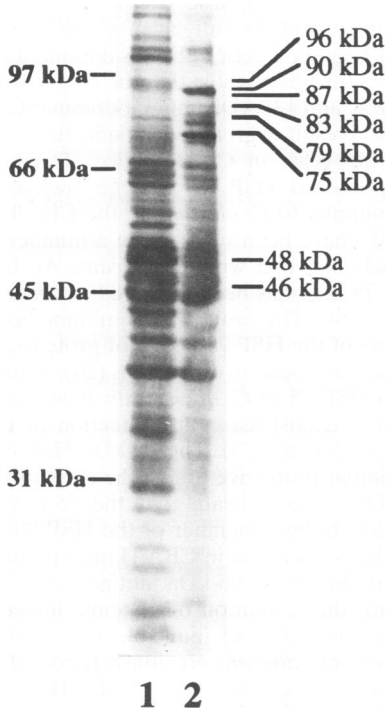

FIG. 3. Direct autoradiograph of immunoblot of antigen extracts of heat-stressed and unstressed *C. albicans* that have been fractionated by SDS-PAGE. The immunoblot was screened with day 24 CBA/H immune sera and probed with alkaline phosphatase-conjugated anti-mouse immunoglobulin (gamma and light chain specific). Lanes: 1, antigen extract from unstressed *C. albicans*; 2, antigen extract from heat-stressed *C. albicans*.

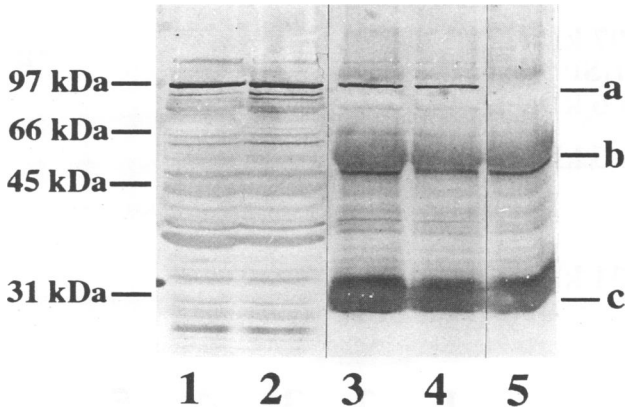

FIG. 4. Immunoblot of extracts from heat-stressed and unstressed *C. albicans* that have been fractionated by SDS-PAGE. The extracts have been immunoprecipitated with protein A-Sepharose and day 24 immune sera from CBA/H mice. The immunoblot was screened with day 24 immune sera from CBA/H mice and probed with alkaline phosphatase-conjugated anti-mouse immunoglobulin (gamma and light chain specific). Lanes: 1, antigen extract from heat-stressed *C. albicans*; 2, antigen extract from unstressed *C. albicans*; 3, immunoprecipitated antigens from an extract of heat-stressed *C. albicans*; 4, immunoprecipitated antigens from an extract of unstressed *C. albicans*; 5, protein A-Sepharose with day 24 CBA/H immune sera.

An additional control sample contained immune sera from CBA/H mice which were incubated with protein A-Sepharose in the absence of *C. albicans* extract. The serum proteins associated with the protein A-Sepharose were separated by SDS-PAGE, electroblotted onto nitrocellulose, and immunostained with CBA/H immune sera. Significant staining was observed with bands of 50 and 29 kDa (Fig. 4, lane 5, positions b and c, respectively). Staining of the bands in the same locations was demonstrated in the other test samples (Fig. 4, lanes 3 and 4, positions b and c). The secondary antibody used for immunostaining was specific for both light and heavy chains of murine IgG. It is likely that the 29- and 50-kDa proteins are the light and heavy chains of mouse IgG, originating from the immune sera.

DISCUSSION

The reactivities of antibodies in sera from CBA/H mice to antigens of *C. albicans* were investigated by Western blotting and immunostaining. Strong antibody reactivities to antigens of 46, 48, 75, 79, 87, 96, and 138 kDa (Fig. 2) were detected. The greatest reactivity, as judged by the intensity of staining, was against the 96-kDa antigen. An HSP from the HSP 90 family is immunodominant in several fungal infections, including candidiasis (14). Investigations were performed to determine whether the immunodominant antigens of *C. albicans* recognized by infected CBA/H mice were HSPs.

Antibodies in the immune mouse serum reacted with an antigen of 96 kDa present in extracts from both unstressed and stressed *C. albicans* (Fig. 2, lanes 1 and 2, respectively). Intense staining of the 96-kDa antigen of *C. albicans* (Fig. 2, lane 1) indicates that the CBA/H mice produce significant amounts of antibody to this antigen. Less intense staining was detected when the mouse serum was reacted with antigens in the extract from heat-stressed *C. albicans* (Fig. 2, lane 2). This suggests that the synthesis of the 96-kDa protein is down regulated during the stress response of *C. albicans* cells. Commonly, both prokaryotic and eukaryotic cells respond to heat stress by increasing the rate of synthesis of HSPs (9). However, radiolabelling of the 96-kDa protein was not detected after autoradiography of radiolabelled proteins in the extract from heat-stressed *C. albicans*. It was concluded that the 96-kDa protein was not induced by heat stressing of the yeast cells (compare Fig. 2, lane 2, with Fig. 3, lane 2). A strongly radiolabelled protein band of 90 kDa was detected by autoradiography of the extract from radiolabelled *C. albicans* (Fig. 3, lane 2); this is likely to be HSP 90 of *C. albicans*. The 90-kDa HSP of *C. albicans* was not reactive with serum from the CBA/H mice previously infected with *C. albicans* (Fig. 2, lane 2).

A 47-kDa breakdown product of HSP 90, characterized by Matthews and Burnie (11), showed 90% sequence identity (98% sequence similarity) to amino acid positions 316 to 709 of *Saccharomyces cerevisiae* HSP 90. Several epitopes recognized by human sera have been identified in this region of *C. albicans* HSP 90 (14). These epitopes are conserved in the corresponding region of *S. cerevisiae* HSP 90. The relationship between the 96-kDa antigen of *C. albicans* and HSP 90 of *S. cerevisiae* is yet to be determined.

An antigen of 75 kDa was reactive with immune CBA/H mouse serum but was less intensely stained than the 96-kDa antigen (Fig. 2). The 75-kDa protein was more intensely radiolabelled in the extract from heat-stressed cells of *C. albicans* than in the unstressed control (Fig. 3, lane 2). It was concluded that the 75-kDa antigen was an HSP.

The 75- and 96-kDa antigens were immunoprecipitated with immune serum from the CBA/H mice. The 75-kDa protein precipitated from extracts prepared from heat-stressed cells of

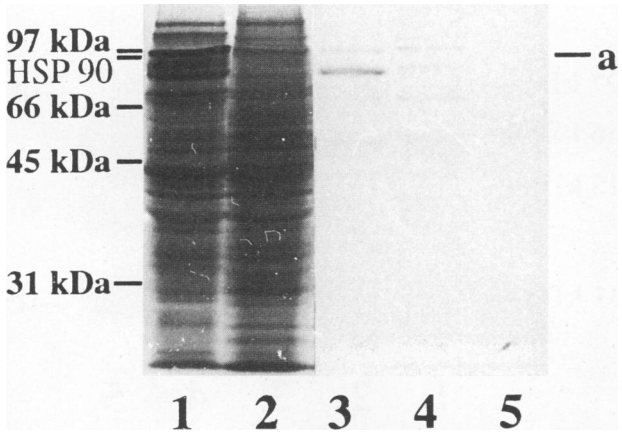

FIG. 5. Direct autoradiograph of immunoblot of extracts from heat-stressed and unstressed *C. albicans* that have been fractionated by SDS-PAGE. The extracts have been immunoprecipitated with protein A-Sepharose and day 24 immune sera from CBA/H mice. The immunoblot was screened with day 24 immune sera from CBA/H mice and probed with alkaline phosphatase-conjugated anti-mouse immunoglobulin (gamma and light chain specific). Lanes: 1, antigen extract from heat-stressed *C. albicans*; 2, antigen extract from unstressed *C. albicans*; 3, immunoprecipitated antigens from an extract of heat-stressed *C. albicans*; 4, immunoprecipitated antigens from an extract of unstressed *C. albicans*; 5, protein A-Sepharose with day 24 CBA/H immune sera.

C. albicans was radiolabelled, whereas the 96-kDa antigen was not (Fig. 5, lanes 3 and 4). The precipitated proteins were reactive with antibodies in the immune serum (Fig. 4, lanes 3 and 4). This confirmed that the 96-kDa antigen was not an HSP whereas the 75-kDa antigen was an HSP.

Passively administered antibodies to HSP 90 of *C. albicans* protected BALB/c mice against lethal infections with the yeast (14). Mice were injected with either sera or the immunoglobulin fraction from two convalescent patients who had antibodies to the 47-kDa breakdown product of HSP 90 of *C. albicans*. Protective immunity was also achieved by using a passively administered murine monoclonal antibody reactive with an epitope of HSP 90. However, in CBA/H mice the antibody response is not directed against HSP 90 of *C. albicans*. Therefore, HSP 90 of *C. albicans* cannot be involved in protective humoral immunity in CBA/H mice. Evidence for a protective effect(s) of antibodies produced by the CBA/H mice against the immunodominant 96-kDa antigen or the 75-kDa HSP is currently being investigated.

The 47-kDa breakdown product of HSP 90 (11) and the 48-kDa enolase (6) are immunodominant antigens that have been identified during human candidiasis. In CBA/H mice the 96-kDa antigen is immunodominant. These immunodominant antigens may have a common function in stimulating the immune response to other *C. albicans* antigens. Mamula and Janeway (10) propose that responses to immunodominant antigens facilitate the diversification of both T-cell and B-cell responses during infection. Helper CD4 T cells primed to immunodominant peptides on dendritic cells in turn activate antigen-binding B cells. These antigen-binding B cells activated by the primed antigen-specific helper T cells are competent to prime naive CD4 T cells. Antigen-specific B cells internalize large amounts of protein and thereby present a greater range of peptides from the original protein to these naive CD4 T cells. Therefore, the antigen that binds to the B cell can diversify the CD4 T-cell response (10). Thus, the major role of the antibody response of the CBA/H mice to the immunodominant 96-kDa antigen of *C. albicans* may be the generation of immunoreactivity to less dominant antigens such as HSP 75. It is further possible that the responses to the other 46-, 48-, 79-, 87-, and 138-kDa immunodominant antigens of *C. albicans* (Fig. 1) result in greater diversification of the humoral response to the yeast in CBA/H mice. The less dominant immune response to HSP 75 may be responsible for the protective immunity to *C. albicans* in the CBA/H mice. Antibodies to HSPs have been reported in a number of microbial infections, including that with *C. albicans*. An immunodominant HSP of 75 kDa has been observed in *Chlamydia trachomatis* infections (4). The protein is membrane bound and, like other members of the HSP 70 family of proteins, may assist in the export and translocation of proteins (20). Serum antibodies in patients to HSP 75 of *C. trachomatis* have been associated with protection against ascending infection of the Fallopian tube with *C. trachomatis* (3). The 75-kDa HSP of *C. albicans* may have a similar protective role in the resolution of systemic candidiasis. Our data indicate that the 75-kDa HSP of *C. albicans* is likely to be a member of the HSP 70 family.

This study has shown that in CBA/H mice immune reactivity to an immunodominant 96-kDa antigen of *C. albicans* is associated with the resolution of systemic infection. The 96-kDa protein is not heat shock inducible and is distinct from the HSP 90 antigen of *C. albicans* previously reported by Matthews et al. (14). Antibody reactivity to a 75-kDa HSP of *C. albicans* in CBA/H mice was also demonstrated.

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