INFECTION AND IMMUNITY, June 1985, p. 806–812
0019-9567/85/060806-07$02.00/0
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Vol. 48, No. 3

Cross-Reacting Human and Rabbit Antibodies to Antigens of *Histoplasma capsulatum*, *Candida albicans*, and *Saccharomyces cerevisiae*

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Received 11 March 1985/Accepted 14 March 1985

Using Western blots of electrophoretically separated antigens, we show that human antibodies react most frequently to antigens shared by three fungi (*Histoplasma capsulatum*, *Candida albicans*, and *Saccharomyces cerevisiae*). Reactivity to antigens specific for individual fungi was relatively uncommon. The pattern of reactivity could not distinguish infected patients from uninfected controls. Rabbits immunized with extracts of each fungus also produced antibodies to cross-reactive or shared antigens of the other two fungi. Furthermore, preimmune sera showed similar but lower reactivity with the same fungal antigens. We believe that the preimmunization antibodies, which probably resulted from earlier fungal colonization or inapparent infections, predisposed the immune responses elicited by the vaccinations. A similar mechanism likely explains the results with human sera.

There has been considerable interest in host defenses and immunological reactivity to fungal infections, which have been increasing for several years (10, 31). The study of these processes is made more difficult by the ability of fungi to cause infections in the presence of both antibody and cellular immunity (2).

The reagents used to study immune responses in fungal infections in most reported studies have been relatively crude mixtures of proteins and carbohydrates derived from autolysed fungal cells (27). In addition, a variable level of cross-reactivity among antigens from different fungi has been reported (7, 14, 30) but not analyzed in detail.

The identification of those components of a fungus which elicit antibody production during the course of an infection might contribute to a better understanding of disease and host protective mechanisms. In addition, identification and isolation of specific antigens in fungi which elicit antibody production may lead to the development of more useful diagnostic tests of infection and perhaps even to molecularly characterized vaccines.

Recently, several techniques for the identification of antibody responses to electrophoretically separated antigens have been developed (4, 9, 14, 16–18, 30, 32). In the work presented here, we show that although the intensity was variable, the most frequent human antibody reactions were to antigens shared by three disparate fungi, *Histoplasma capsulatum*, *Candida albicans*, and *Saccharomyces cerevisiae*.

The specificity of rabbit antibodies deliberately elicited by cell extracts of *H. capsulatum* or *C. albicans* was similar to that of human antibodies. Since antibodies to the same fungal antigens were already present in very low amounts in rabbit preimmune sera, we infer that both the rabbit and human immune systems are repeatedly stimulated by fungal antigens, because fungi are ubiquitous in the environment and often colonize various parts of the body. This repetitive exposure stimulates an immune response and conditions the host to subsequent immune challenges even in the absence of disease.

MATERIALS AND METHODS

Collection of sera. Blood was drawn from the antecubital vein of normal volunteers and patients and allowed to clot. Serum was separated from the clot by centrifugation and stored in 0.5-ml volumes at −70°C. The serum was thawed just before use in the experiments and was not refrozen.

The patients with histoplasmosis had varied clinical presentations and were all in the acute or subacute stage of their infection. They all had positive serological determinations by immunodiffusion (H and M bands) and complement fixation. The diagnoses were confirmed by positive cultures. The patients varied in age from 23 to 71 years. The normal donors were healthy at the time of the bleeding and had no history of histoplasmosis. Chest X-rays were all normal.

Organisms. *H. capsulatum* (Downs strain), *C. albicans*, and *S. cerevisiae* were obtained from our laboratory culture collection. All organisms were grown to late log phase in shaking water baths in broth containing 2% glucose and 1% yeast extract. For *C. albicans* and *S. cerevisiae*, the temperature of incubation was 25°C. *H. capsulatum* was grown in the yeast phase at 37°C. This growth usually took 48 h for *S. cerevisiae*, 48 h for *C. albicans*, and 72 h for *H. capsulatum*. After the stated periods of growth, the cells were collected by centrifugation at 2,500 × *g*. The cells were then extensively washed with deionized water and kept frozen at −70°C until they were ready to be broken.

Cell fractionation. The organisms were suspended in a "breaking" buffer and broken by mechanical disruption in a Braun homogenizer for 2 min with intermittent cooling in the presence of glass beads (0.45-mm diameter). The "breaking" buffer used was made up of 62.5 mM Tris-hydrochloride (pH 6.8) containing 15% glycerol, 1 mM dithiothreitol, and 0.2 mg of phenylmethylsulfonyl fluoride per ml. After disruption of the cells, the glass beads and unbroken cells were removed by centrifugation at 500 × *g*. The homogenate was then centrifuged at 6,000 × *g* for 20 min, and the pellet or cell envelope was collected. The supernatant was centri-

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fuged at 26,000 × g in a Sorvall centrifuge; this pellet was the mitochondrial fraction. The mitochondrial supernatant was centrifuged again at 105,000 × g; the pellet was the microsomal fraction, and the supernatant was the soluble fraction or high-speed supernatant. These fractions were obtained for *H. capsulatum* (yeast phase) and *C. albicans* (yeast phase); only the envelope and microsomal fractions of *S. cerevisiae* were used in our experiments.

Rabbit immunization. New Zealand rabbits weighing 1.5 to 2.0 kg were first bled to obtain preimmune sera and then immunized with homogenates of yeast cells of *H. capsulatum* or *C. albicans*. The cell homogenates were prepared by breaking the organisms in phosphate-buffered saline (PBS) containing 0.1% Nonidet P-40 (NP-40). After the cells were broken with glass beads in a Braun homogenizer, the homogenate was separated from unbroken cells by centrifugation at 1,500 × g for 15 min. The supernatants of the cell homogenate were mixed with sodium dodecyl sulfate (SDS) and NP-40 to a final concentration of 0.1% each, and 10-mg samples in 1 ml of PBS were mixed with 1 ml of complete Freund adjuvant (Cappel Laboratories, West Chester, Pa.). The rabbits received an initial injection of 0.25 ml of the mixture in each footpad, followed by booster injections subcutaneously at several sites every week for 3 weeks. They were then rested for 1 week after which time they were bled to obtain immune serum. The antibody reactivity of preimmune and immune sera was determined by agar gel diffusion. One percent nonionic detergent (NP-40) was used to extract crude whole cell homogenates for the antigens used in the agar gel diffusion.

Protein estimation. Protein in each subcellular fraction was estimated with a reagent from Bio-Rad Laboratories (Richmond, Calif.), using bovine serum albumin (BSA) as the standard.

Polyacrylamide gel electrophoresis. Breaking buffer (15 μl) containing 30 μg of protein from each cell fraction was mixed with an equal volume of 62.5 mM Tris-hydrochloride (pH 6.8) containing 5% SDS, 2% β-mercaptoethanol, and 15% glycerol, boiled for 2 min, cooled, and centrifuged. The supernatant fractions were electrophoresed as described by Laemmli (21). Standard markers were a mixture from Pharmacia Fine Chemicals, Uppsala, Sweden. The molecular weights of the antigens were determined as described previously (28).

Transfer of electrophoretically separated antigens to nitrocellulose papers. After the electrophoresis was completed, proteins were transferred to nitrocellulose paper (pore size, 0.45 μm; Millipore Corp., Boston, Mass.) in an electrophoretic transfer chamber (Hoefer Scientific, San Francisco, Calif.) as described earlier (6). Transfer efficiency, determined by optical scanning of Coomassie blue-stained gels, was 30 to 50% for every band.

^{125}I-labeling of protein A was done by the chloramine T method as described by Dorval et al. (12). The unreacted ^{125}I was removed by Sephadex G-25 column chromatography.

Detection of antigen-antibody complexes on nitrocellulose papers was done by a modification of an earlier procedure (29). The nitrocellulose papers with the antigens transferred from the polyacrylamide gels were saturated for 5 h with 5% BSA in PBS containing 0.1% NP-40 at 37°C and then were reacted with rabbit serum (1:100 dilution in a total of 10 ml of PBS containing 5% BSA and 0.1% NP-40) for 16 h at room temperature. After washing, the papers were again transferred to plastic bags and incubated in 10 ml of PBS containing 5% BSA, 0.1% NP-40, and 8 × 10^6 cpm of ^{125}I-protein A (20 × 10^6 cpm/mg) for 1 h at room temperature. Under these conditions, ^{125}I-protein A did not bind to the fungal antigen in the absence of antibody. In several experiments, ^{125}I anti-rabbit goat serum was used in the place of ^{125}I-protein A. Specific activities of anti-rabbit sera ranged from 4 × 10^6 to 6 × 10^6 cpm/mg of protein. Portions of the sera containing 8 × 10^6 cpm were used for detection of antigen-antibody complexes. After incubation, the papers were taken out of the bags, washed with PBS containing 0.1% NP-40, dried, and subjected to autoradiography.

Adsorption experiments. In the adsorption experiments with rabbit or human sera, equal volumes of test serum and rabbit antibody to human skin collagenase (a gift from Eugene Bauer, Dermatology Division, Washington University School of Medicine, St. Louis, Mo.) were mixed. One 200-μl portion of this serum mixture was incubated with 200 μl of a cell homogenate of *C. albicans*, and another was incubated with 200 μl of a cell homogenate of *H. capsulatum* overnight in the cold. The antiserum-antigen mixtures were centrifuged, and the supernatants were again incubated with cell homogenate fractions of *C. albicans* or *H. capsulatum* for 4 h at 4°C. The final supernatants containing antibodies after adsorption with extracts of *C. albicans* or *H. capsulatum* were brought to overall dilutions of 1:100 in 10 ml with PBS containing 5% BSA and 0.1% NP-40 and used to detect the antigens. The unadsorbed sera in these experiments were handled in the same manner, except that identical volumes of the buffer instead of the cell homogenates were used as the adsorbant. In the experiments with preimmune rabbit serum, the serum was mixed with an equal volume of rabbit antibody to human skin collagenase. A 200-μl portion of the mixture was diluted 1:100 in 10 ml of PBS containing 5% BSA and 0.1% NP-40 and used to detect the antigens. In the experiments in which adsorbed sera or the preimmune sera were used, one corner of the cellulose nitrate paper containing the antigens was spotted with different dilutions of 10 μg of human skin collagenase in 5 μl. Proportional reactivity with the spotted collagenase ruled out the possibility of nonspecific adsorption of antibody or inactivation of the serum.

Immunoprecipitation. Immunoprecipitation of the antigens with antisera was done by a modification of a procedure described earlier (25). The protein concentration of the antigen in the supernatant was adjusted to 10 mg/ml with PBS and labeled with ^{125}I by the chloramine T method described above. A 30-μl portion of the specified antiserum was mixed overnight with 5 × 10^6 cpm of the antigen in a total volume of 500 μl at 4°C with constant rocking. Then, 10 mg of protein A-Sepharose beads, previously swollen in RIP buffer (25 mM Tris [pH 7.2], 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.9% sodium chloride, and 1 mM EDTA) was then added to the antigen-antiserum mixture and left at room temperature (25°C) with constant rocking for 2 h. The Sepharose beads were then removed by centrifugation in a Microfuge, washed four times with ice-cold RIP buffer, boiled for 3 min in sample buffer (62.5 mM Tris-hydrochloride [pH 6.8], 2.5% SDS, 0.001% bromophenol blue, 0.1% sodium deoxycholate, 2% β-mercaptoethanol, and 15% glycerol), cooled, and centrifuged again in a Microfuge for 2 min. The supernatant was electrophoresed in a 12% polyacrylamide gel as described above. After electrophoresis, the gel was dried and subjected to autoradiography.

RESULTS

Electrophoretic patterns of the fungal antigens. Figure 1 shows protein bands of *H. capsulatum*, *C. albicans*, and *S.*

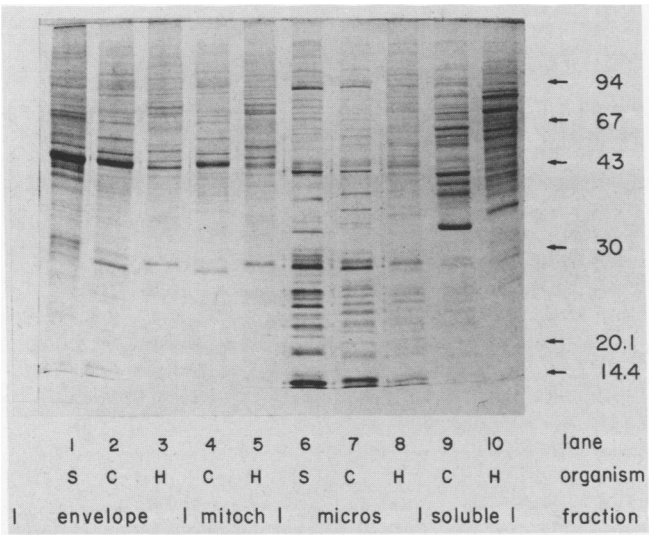

FIG. 1. SDS-polyacrylamide gel analysis of the antigens from different fractions of *S. cerevisiae* (S), *C. albicans* (C), and *H. capsulatum* (H). Samples (5 μg) of protein from envelope, mitochondrial, microsomal, and high-speed supernatant (soluble) fractions of the fungi were solubilized, electrophoresed, and stained as described in the text.

cerevisiae cellular fractions separated by SDS-polyacrylamide gel electrophoresis. Molecular weights ranged from 100,000 to 14,000. The most intensely stained bands were different in each of the fractions but had similar migrations in the same fractions of each of the fungi.

Reactions of sera from patients with histoplasmosis with the fungal antigens. Figure 2a shows the pattern of reactivity of serum from a patient with histoplasmosis with the fungal antigens. Antigen-antibody complexes on the paper were detected with ^{125}I-labeled protein A. The envelope and mitochondrial fractions of *H. capsulatum* showed the highest level of reactivity, too high to resolve bands easily (Fig. 2a, lanes 3 and 5), but less reactive bands were also seen in the microsomal and soluble fractions (lanes 8 and 10, respectively). Reactivity against the same fractions of *S. cerevisiae* and *C. albicans* was relatively weak, but in every case the most intense reactivity was to bands ranging in molecular weight from 94,000 to 35,000. The diffuse reactivity of the serum in lanes 3 and 5 may be due to antigenically active nonprotein groups (lipids or carbohydrates) attached to the fungal proteins.

When the serum from the patient was first adsorbed with sonic extracts of *C. albicans* before reaction with the fungal antigens, there was a considerable decrease in the reactivity in all of the lanes (Fig. 2b). Reactivity of the adsorbed serum was no longer apparent in the lanes with the envelope and mitochondrial fractions of *S. cerevisiae* and *C. albicans*; it was still present, although markedly decreased, against bands in the lanes with the antigens from *H. capsulatum*. Control antibody to human skin collagenase mixed with sera was not decreased by the adsorption.

Serum from a second patient with histoplasmosis had a large number of reactive bands of low intensity in every fraction of the three fungi (Fig. 3a). However, intensely reactive antigens were present with molecular weights of 100,000 to 68,000 in the envelope and mitochondrial fractions of *C. albicans* (Fig 3a, lanes 2 and 4). A high-molecular-weight band, larger than 100,000 in the envelope and soluble fractions of *H. capsulatum*, also reacted with the serum. As in the other patient, many of the reactive bands in the three fungi comigrated, and the general pattern of reactivity of the serum against the antigen in all three fungi was similar.

When the serum from this patient was adsorbed with sonic extracts of either *C. albicans* or *H. capsulatum* before the reaction with the fungal antigens, almost all of the reactive bands in all three of the fungi disappeared (Fig. 3b). Here again, the adsorption appeared to be specific since it did not decrease reactivity to different dilutions of collagenase.

The reactivities of sera from 25 additional patients with documented histoplasmosis were tested in the same way. All of the patients reacted to at least one antigen in molecular weight ranges of 100,000 to 94,000, 80,000, 68,000, 46,000 and 35,000 (Table 1). In general, the patterns of reactivity of individual patients to the antigens of all three fungi were

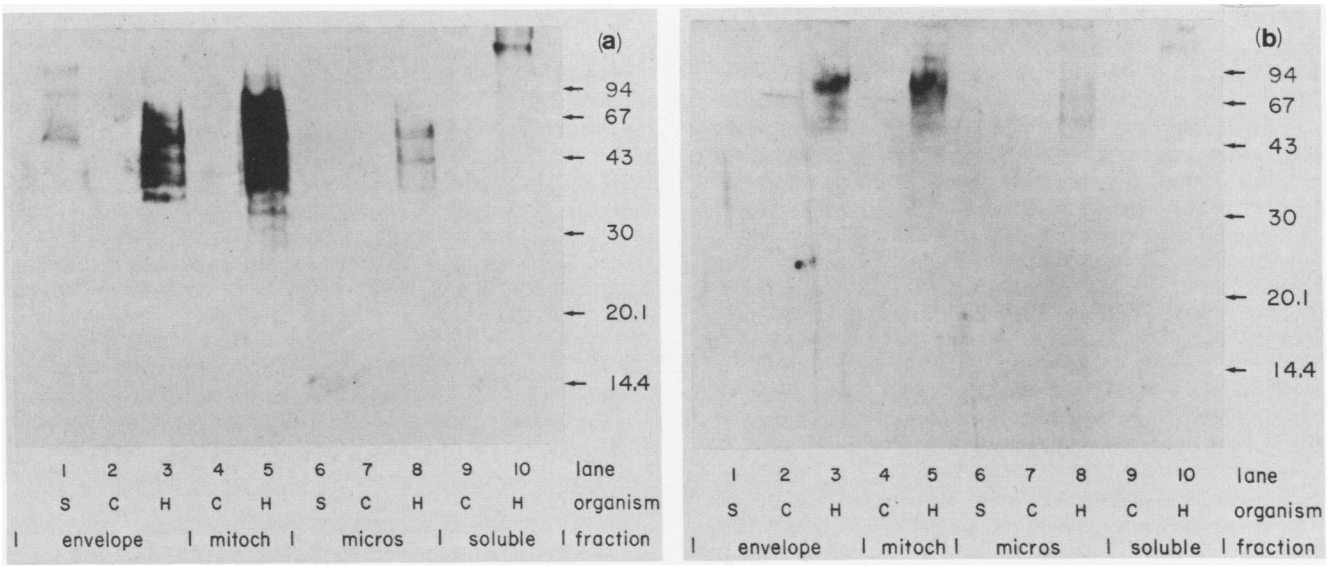

FIG. 2. Reactivity of serum from a patient with histoplasmosis. The reactivity of the serum before (a) and after (b) adsorption with the extracts of *C. albicans* was determined. Abbreviations are the same as in Fig. 1.

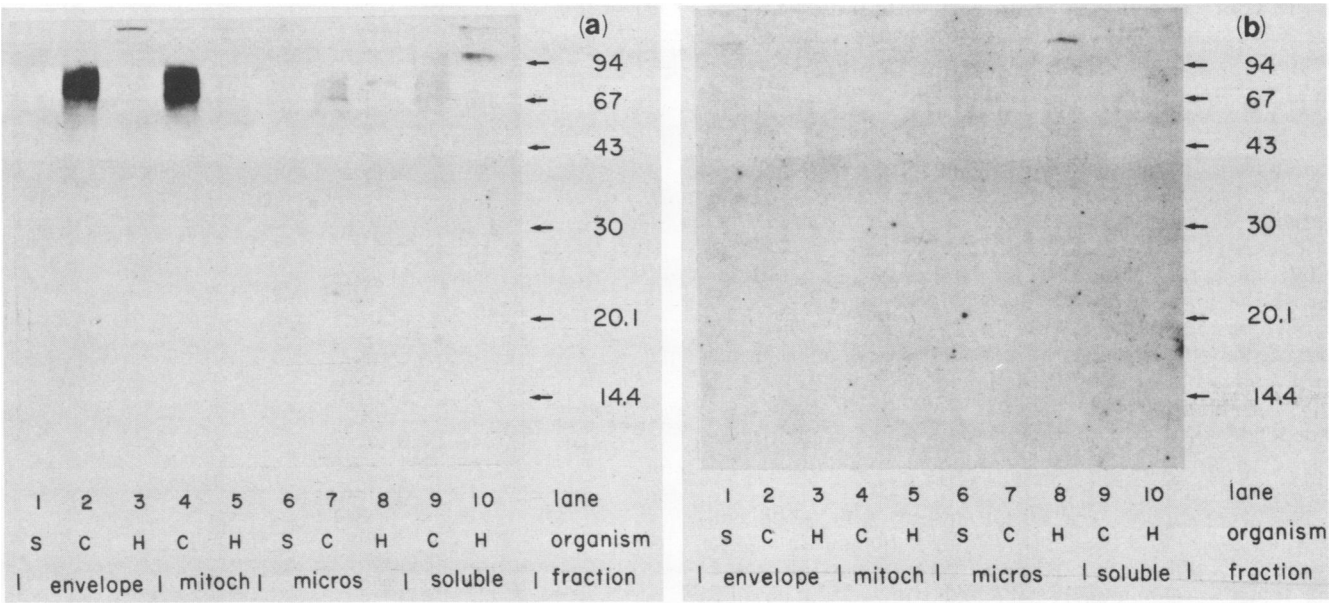

FIG. 3. Reactivity of the serum from a patient with histoplasmosis. The reactivity of the serum of the patient to the antigens of the fungi before (a) and after (b) adsorption with extracts of *C. albicans* was determined. Abbreviations are the same as in Fig. 1.

very similar, though the intensity of the reactions varied. Fifteen of the patients reacted more intensely to antigens from *C. albicans*; 12 reacted more intensely to antigens of *H. capsulatum*. The antigens of *S. cerevisiae* were the least reactive. Sera generally reacted more to the envelope and mitochondrial fractions. There were nine patients who reacted to bands that were found only in fractions of *H. capsulatum* or *C. albicans*. However, when the sera were preadsorbed with sonic extracts of *C. albicans*, the reactivity to these bands as well as to the comigrating bands in the other fungi disappeared or was considerably reduced. The patterns of reactivity showed no correlation with the seriousness of the infections, clinical status of the patients, or stage of the disease.

We tested the reactivity of sera from eight normal volunteers without clinical history of histoplasmosis. Six of the donors had lived in the St. Louis area for many years (three had positive histoplasmin skin tests and three did not), and two had only recently arrived in the St. Louis area from areas of the world in which histoplasmosis was not endemic (Union of South Africa; Central India). The latter two donors had negative histoplasmin skin tests (skin tests were done after the sera were obtained).

TABLE 1. Reactivity of sera from patients with histoplasmosis and from normal volunteer donors with antigens from three fungi

Mol wt (kd) of antigen	Reactivity[a] to antigens of:					
	S. cerevisiae		*C. albicans*		*H. capsulatum*	
	Patients	Normal volunteers	Patients	Normal volunteers	Patients	Normal volunteers
100–94	20	7	25	6	24	5
80	6	1	23	5	21	2
68	9	1	26	4	12	3
60	3	1	10	1	10	
55	4	2	9	3	11	3
46	17	6	19	5	20	3
40	6	2	10		12	3
38	9	4	10	1	11	2
35	19	2	22	6	21	5
28	12	2	10	3	8	5
25	16	2	15	3	12	1
20	12	2	10		4	1
18	9		9	1	4	2

[a] The total number of patients tested was 27, and there were 8 volunteers with no previous history of histoplasmosis. Of the eight volunteers, three were histoplasmin skin test positive. Numbers represent the number of patients reacting to the antigens with the specified molecular weights.

The patterns of reactivity of these control sera (Table 1) did not differ from those of sera from patients (though all reacted most strongly against antigens of *C. albicans*).

Reactions of sera from immunized and nonimmunized rabbits to fungal antigens. Because human sera showed-cross reactivity to fungal antigens, we tested sera from rabbits immunized with extracts of either *C. albicans* or *H. capsulatum* to determine whether a similar pattern of reactivity to the fungal antigen was present.

Figure 4 shows the reactions of serum from a rabbit immunized with sonic extracts of *H. capsulatum*. The strongest reactivity was to antigens of *H. capsulatum*, and at least two bands were unique to fractions of this fungus, an 18-kilodalton (kd) band (in the envelope and mitochondrial fractions) and a 14.4-kd band (in the envelope, mitochondrial, and microsomal fractions). However, there were also strongly reactive bands in fractions of the other two fungi, most of which comigrated with bands in the *H. capsulatum* fractions. Again, as with human sera, antigens from *C. albicans* reacted more intensely with the antisera than did antigens from *S. cerevisiae*.

Serum from a rabbit before immunization with *H. capsulatum* did not react with any of the fungal antigens. However, when the serum was mixed with rabbit antibody to human collagenase, the reactions of the mixture to various dilutions of the collagenase were distinct.

Serum from a rabbit immunized with *C. albicans* was also reacted with the fractions of the three fungi (Fig. 5). The highest level of reaction of this serum was to high-molecular-weight antigens of *C. albicans*.

Reactivity to all of the fungal fractions was decreased when the serum was first adsorbed with extracts of *C.*

albicans or *H. capsulatum*. In each case, adsorption decreased reactivity against all fungal antigens. Reactivity of control antibody to human skin collagenase, however, was not decreased by adsorption with extracts of *C. albicans* (data not shown).

The experiments shown in Fig. 4 and 5 used ^{125}I-protein A to determine bound antibody; similar results were obtained when ^{125}I-labeled anti-rabbit goat serum was used for this purpose (data not shown).

Reactivity of the sera with specific antigens was not related to antigen concentration, size, or efficiency of transfer of the antigens to the nitrocellulose paper, since the bands present in the lowest amounts, as determined by Coomassie blue staining, were frequently the most prominent on autoradiography. Reactions also varied proportionally with dilutions of the sera and were identical when two independent assays with protein A or goat anti-rabbit serum was used. In addition, under identical conditions, serum from preimmune rabbits did not show reactivity.

Immunoprecipitation of fungal antigens with rabbit antisera. For a study of the cross-reacting antibodies in another system, ^{125}I-labeled nonionic detergent extracts of each of the fungi were immunoprecipitated with serum obtained from nonimmunized rabbits and rabbits immunized with *H. capsulatum* or *C. albicans*. Radiolabeled antigen-antibody complexes were precipitated and then electrophoresed (Fig. 6). As expected, the highest level of reactivity of the serum obtained from animals immunized with *C. albicans* was with the antigens from the homologous fungus (Fig. 6, lane 2). However, this serum also reacted with bands from *S. cerevisiae* (lane 1) and *H. capsulatum* (lane 3). A similar result was obtained when the fungal antigens were reacted with serum from rabbits immunized with *H. capsulatum*. Most cross-reacting bands in the different fungi comigrated with antigens in the homologous reactions (compare lanes 1, 2, and 3 with lanes 4, 5, and 6). In addition, the electrophoretic pattern of the antigens of *H. capsulatum* precipitated by the serum raised by immunization with *C. albicans* was very similar to that seen when the antigens were precipitated

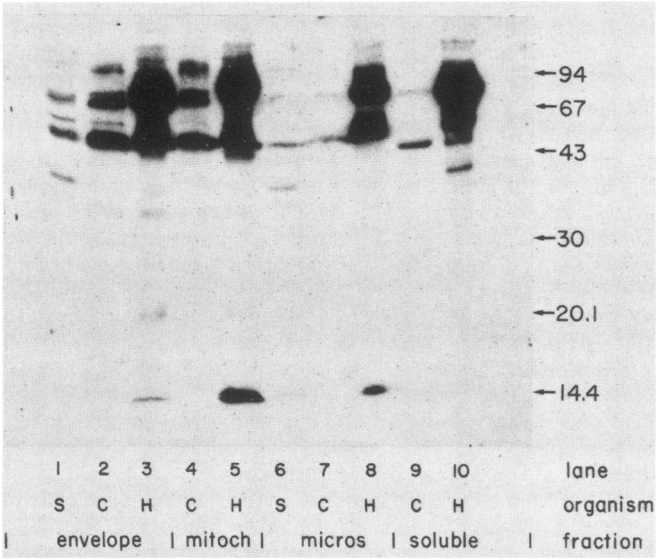

FIG. 4. Reactivity of the serum from a rabbit after immunization with extracts of *H. capsulatum* with the fungal antigens. Abbreviations are the same as in Fig. 1.

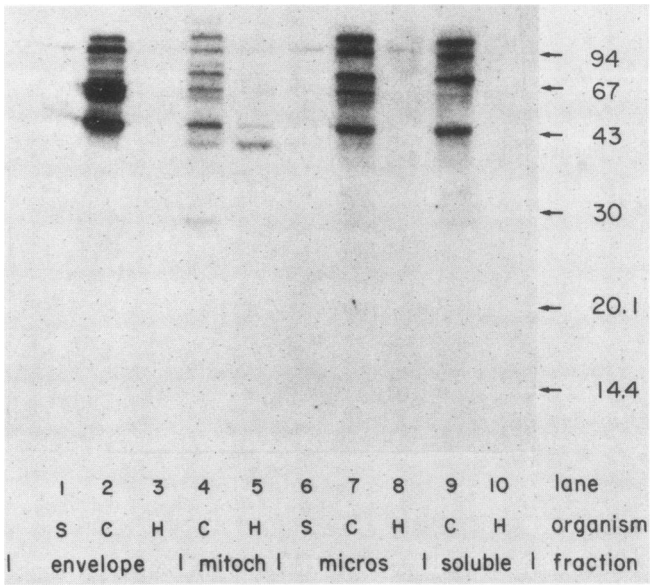

FIG. 5. Reactivity of the serum from a rabbit after immunization with extracts of *C. albicans* to the fungal antigens. Abbreviations are the same as in Fig. 1.

by serum obtained from rabbits immunized with *H. capsulatum* (compare lane 3 with lane 6 in Fig. 6). The same was true for the antigen of *C. albicans* (compare lanes 2 and 5). Therefore, the same antigens were being precipitated from each fungal extract by both homologous and heterologous antisera.

Each of the antisera had different levels of reactivity to the fungal antigens, and the preimmune serum barely reacted (Fig. 6A, lanes 7 to 9). However, when the X-ray films were exposed to the papers for 6 days instead of 2 days (Fig. 6B), the reactivity of the preimmune serum became more apparent, and the patterns obtained were identical to those of the serum from rabbits immunized with *C. albicans*. Therefore, the preimmune rabbits appeared to have low levels of antibodies which reacted with the fungal antigens with the same pattern as the serum from animals immunized with *C. albicans*.

The immunoprecipitation appeared to be specific because fetal bovine serum, used as a negative control, did not react with the fungal antigens.

DISCUSSION

Cross-reacting or common epitopes in the different fungi were detected by antibodies in both human and rabbit sera. The recognition of some cross-reacting or shared epitopes in these fungi was not unexpected; such cross-reactions have been described previously for various organisms, including bacteria (22), viruses (9, 11, 22), fungi (7, 8, 14, 30), parasites (1, 15, 16), and various purified protein antigens (19, 24, 26). In the case of fungi, cross-reactivity has been a recognized problem in serological diagnostic testing, and efforts have been made to characterize these reactions (14, 30). However, it has not been done in the manner we describe, and it was unexpected that the most frequent and most intense reactivity of the sera from the rabbits was not to antigens specific to the immunizing fungus but to the shared antigens of the three fungi. Reactivity to specific fungal antigens did occur, but with low frequency.

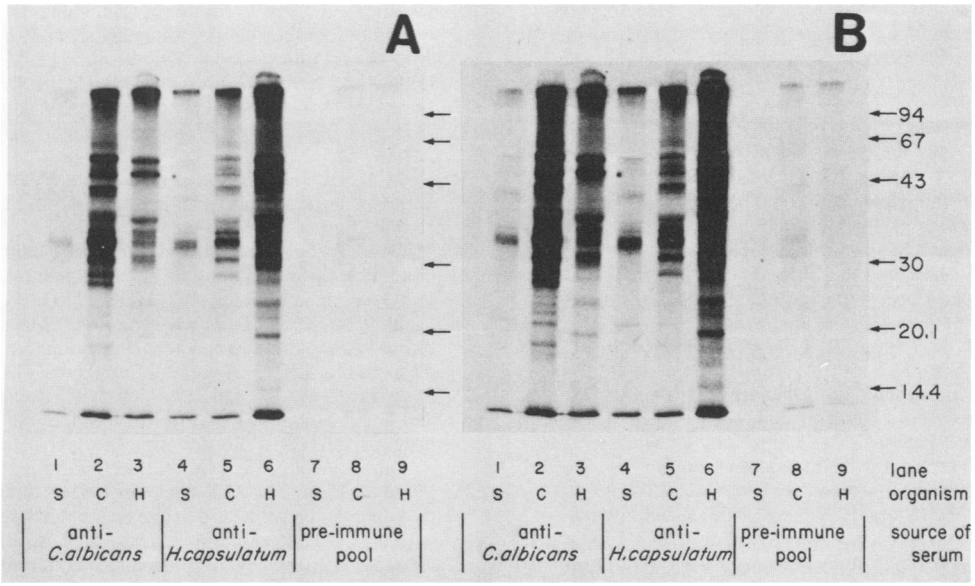

FIG. 6. SDS-polyacrylamide gel analysis of ^{125}I-labeled antigens of *C. albicans* and *H. capsulatum* precipitated with antisera from rabbits immunized with extracts of *C. albicans* (anti-*C. albicans*), *H. capsulatum* (anti-*H. capsulatum*), or preimmune sera (preimmune pool). A, Results after exposure of the X-ray film for 48 h; B, exposure after 6 days. Abbreviations are the same as in Fig. 1.

A likely explanation of our findings is provided by the immunoprecipitation experiment (Fig. 6). Although reactivity of the sera was highest when antigens of the immunizing fungi were used, there was a significant level of cross-reactivity. In addition, serum from preimmune rabbits had low levels of cross-reactivity to antigens of all three fungi. Significantly, reactivity of the preimmune serum to *C. albicans* antigens was highest, and the patterns of reactivity to antigens of all three of the fungi most closely resembled those of the rabbits immunized with *C. albicans*. The rabbits used in these experiments were not germfree. It is likely that they were colonized with *Candida* species, giving rise to the low level of reactivity of the preimmune serum. This constant immunological exposure could then determine the subsequent antibody responses to the fungal antigens by stimulating secondary B-cell proliferation to cross-reacting epitopes. Therefore, our results are consistent with the concept of "original antigenic sin," which states that antibody-forming mechanisms can be highly conditioned by the first stimulus so that later infections or challenge with organisms of the same type successively enhance the original antibody (13). This hypothesis was formulated by Francis (13) to explain age-related antibody responses to influenza virus and has recently been discussed as an explanation for human immune responses to structurally defined bacterial polysaccharide antigens (3). In the case of *C. albicans*, colonization or subclinical infection with this common component of normal flora probably determined subsequent antibody responses to fungal antigens.

"Original antigenic sin" could provide some host resistance to fungal infections. Perhaps this is one reason that systemic fungal infections occur uncommonly and, in the absence of immunosuppression, are frequently self-limited.

Will the high frequency of responses to shared fungal antigens prevent the isolation of diagnostic reagents which will be highly specific in serological tests for fungal infection? For example, if antibodies usually arise to shared antigens, and specific reactions are of low intensity and infrequent, the former are too nonspecific and latter to insensitive for use. In addition, the absence of patterns of reactivity to differentiate infected patients from normal controls further complicates our efforts to develop more useful diagnostic tests. Possibly, the cross-reacting or shared antigens themselves offer promise as diagnostic reagents because of the high frequency and intensity of the reactions. Since most proteins possess several different antigenic domains (19), it is possible that specific epitopes are present as part of these shared antigens and therefore could be used as diagnostic reagents. Preliminary studies with monoclonal antibodies produced from splenocytes of mice immunized with the mitochondrial fraction of *C. albicans* support this hypothesis (B. V. Kumar, unpublished data).

ACKNOWLEDGMENTS

This research was supported by the Public Health Service Program in Medical Mycology under grant AI 16228 and by Public Health Service training grants AI 07172 and AI 07015, all from the National Institute of Allergy and Infectious Diseases, and by a fellowship from the South African government (W.L.S.).

We are very grateful for the helpful advice and discussions of our work with Joseph Davie, J. R. Little, Dan L. Longo, Richard Markham, David Schlessinger, and Gary Weil.

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