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Enhanced Antibody Responses Induced by *Candida albicans* in Mice

JIM E. CUTLER* AND RUTH K. LLOYD

Department of Microbiology, Montana State University, Bozeman, Montana 59717

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Candida albicans may immunopotentiate antibody responses in mice to antigens unrelated to the fungus. This effect occurred best with cell-associated, rather than soluble, antigens. When dead yeasts, cell walls, or a water-soluble candidal polysaccharide were used, immunopotentiation was most dramatic when the antigen and fungal materials were given concomitantly via an intraperitoneal injection. However, mice infected with viable yeasts several days before antigen administration also developed heightened responses to the antigen. The mechanism of the *C. albicans*-induced adjuvanticity was not defined, but the effect seemed to correlate with induction of inflammation. The presence of *C. albicans* or other inflammatory agents in the peritoneal cavity caused a more rapid uptake of particulate antigen by the liver. The relationship between this event and immunopotentiation is not known. These studies demonstrate that *C. albicans* may have profound effects on host immune responses. Because immunological aberrations are commonly found in patients with candidiasis it may be important to determine whether some of these aberrations result from, rather than precede candidiasis.

Host defense mechanisms against the opportunistic fungal pathogen *Candida albicans* have not been well defined. There are numerous reports of clinical observations relating various immune and endocrine dysfunctions with susceptibility to candidiasis, and controlled experiments have been done in animal models which simulate human candidiasis. Yet the literature is replete with conflicting data. Clinical observations have led to the general feeling that cell-mediated immune function is a critical host defense against chronic mucocutaneous candidiasis (12), but many individuals with chronic mucocutaneous candidiasis do not have detectable cell-mediated immune defects (36) and T-cell-deficient animals are not more susceptible to acute candidiasis (2, 29). Because antibodies against *C. albicans* are commonly found in patients with various forms of candidiasis it has been held that the specific fungal antibodies are not protective, but indirect evidence that such antibodies may be protective has been obtained by using the mouse as an animal model (22, 24). Numerous reports have shown that human neutrophils and macrophages can ingest and kill *C. albicans* (15, 16, 32), but candidiasis is not necessarily a problem in individuals with defective phagocytic cells.

Classical methods have been used in most of the above studies. That is, in clinical observations a patient with candidiasis is assessed for immune dysfunctions and correlations are drawn. In the controlled experimental situation, various immune functions are either stimulated or suppressed, and then the animals are tested for susceptibility to a viable challenge of the fungus. These experimental protocols are designed primarily to ask what is the effect of various host defense mechanisms on *C. albicans*.

Recently, investigators have begun to ask what is the effect of *C. albicans* on host immunity. Suppressor T-cell activity was found in three of four patients who had relapse of a disseminated fungal, but not candidal, disease (34). Enhanced specific antibody production has been observed in patients with chronic mucocutaneous candidiasis (35). A polysaccharide from *C. albicans* has been reported to induce both human T-cell-dependent B-cell mitogenic responses and, surprisingly, B-cell-dependent T-cell mitogenic responses in vitro (25). Cell walls of *C. albicans* have also been found to enhance delayed-type hypersensitivity reactions to ovalbumin in guinea pigs (13). Alteration of host immune mechanisms by *C. albicans* may explain, in part, some of the apparent controversies regarding host defense mechanisms against candidiasis.

The purpose of this study was to examine the influence of *C. albicans* on mouse spleen plaque-forming cell (PFC) responses to various

nonfungal antigens. Conditions were established under which the fungus acts as an immunopotentiator. Investigations were also initiated to explain the mechanism of immunopotentiation which seems to differ from adjuvant effects by gram-negative rod lipopolysaccharide (LPS).

MATERIALS AND METHODS

Experimental animals. BALB/c mice, originally obtained from Baylor Medical School (Houston, Tex.), were maintained and monitored for sensitization to *C. albicans* as previously described (4). Other mice used in these studies were DBA/1J obtained from Jackson Laboratories (Bar Harbor, Maine) and Swiss-Webster mice originally obtained from Laboratory Supply Co. (Indianapolis, Ind.) and maintained at the Veterinary Research Laboratory of Montana State University. Neither of the latter strains of mice had detectable antibodies against *C. albicans*.

Microbe cultivation. *C. albicans* strain 9938, isolated from a skin lesion of a patient (Medical Mycology Unit, Tulane University), was used in all experiments unless indicated otherwise. The organisms were grown in yeast form under aerobic conditions in 2.0% glucose–0.3% yeast extract–1.0% peptone broth with rotation at 160 rpm (Gyratory Incubator-Shaker; New Brunswick Scientific Co., New Brunswick, N.J.) for 48 to 72 h at 37°C. Fungi were harvested by centrifugation, washed three times in sterile saline, and suspended to the appropriate concentration in saline. In some cases, the yeasts were heat killed (65°C, 10 min) before washing.

Escherichia coli strain O113 was grown in brain heart infusion broth for 24 h under the above conditions. The bacteria were harvested by centrifugation and washed three times in saline.

Yeast cell wall preparation. Cell wall fractions of yeast cells were prepared with a Braun homogenizer (Bronwill Model MSK; VWR, Seattle, Wash.) and differential centrifugation as previously described (3). After fractionation the cell wall preparations were lyophilized and stored in a dessicator until use.

Extraction procedures. Yeast cell wall polysaccharide material was obtained from the aqueous phase of a hot phenol extraction (37) as before (3) and will be referred to as *C. albicans* polysaccharide. LPS from *E. coli* was obtained by the same protocol. Approximately 175 μg of the LPS was lethal to 50% of BALB/c mice within 48 h after intraperitoneal (i.p.) injection.

Antigens used for sensitization. Sheep erythrocytes (SE; in Alsever's; Colorado Serum Co., Denver, Colo.) were washed three times and suspended in sterile 0.15 N sodium chloride (saline) to the appropriate cell concentration before injecting into animals. Polyvinylpyrrolidone (PVP; average molecular weight, 360,000; General Biochemicals Div. Mogul Corp., Chagrin Falls, Ohio) was dissolved in pH 7.2 0.01 M phosphate-buffered saline, and mice were sensitized by injecting 0.25 μg intravenously (i.v.) or i.p. either alone or in combination with 10^7 cells of *C. albicans*.

The effect of *C. albicans* on enhancing anti-chicken gamma globulin (anti-CGG; Cappel Laboratories, Cochranville, Pa.) PFC responses in mice was determined by injecting chicken gamma globulin (CGG; Cappel Laboratories) in soluble form i.p. alone or with the fungus or by injecting CGG-coated horse erythrocytes (HE; Colorado Serum Co.) i.p. alone or with the fungus. CGG was brought to the desired concentration by dilution in phosphate-buffered saline. Coating of HE with CGG was done by a chromium chloride procedure in which 0.5 ml of packed HE, washed three times with saline, were suspended in 1.0 ml of saline containing 1 mg of CGG. One milliliter of 0.1% chromium chloride ($CrCl_3 \cdot H_2O$) in saline was added, the mixture was incubated for 5 min at 22 to 24°C, and the coated erythrocytes were washed four times with saline. In all experiments coating was confirmed by hemagglutinability of the cells upon the addition of anti-CGG. In some experiments estimation of the efficiency of coating was done by adding a known amount of ^{125}I-labeled CGG (iodination of CGG was done enzymatically using the Radioiodination System; New England Nuclear Corp., Boston, Mass.) to the chromium chloride reaction mixture. Approximately 10% of CGG was found to attach to the HE. These calculations indicated that it required about 100 times more soluble CGG to give comparable CGG-specific PFC responses as in mice immunized with the CGG-HE conjugate.

Mouse thyroglobulin was prepared as described elsewhere (8), with some exceptions, as follows. Thyroids were removed from mice and homogenized in ice-cold saline with a hand glass tissue grinder. The homogenate was centrifuged at 350 × *g* for 30 min, and the supernatant material was again centrifuged at 65,000 × *g* for 45 min. The thyroglobulin was precipitated with 45% saturated ammonium sulfate, solubilized in saline, and dialyzed against cold saline to remove residual ammonium sulfate. The solution of thyroglobulin was filter sterilized and stored at −20°C until use. The effect of *C. albicans* on induction of autoantibodies (i.e., anti-thyroglobulin) was determined by immunizing DBA/1J mice with 50 μg of mouse thyroglobulin i.v. (N. Rose, personal communique) with or without adjuvant on days 0 and 7. In this experiment adjuvants consisted of whole cells or fractions of *C. albicans* given i.v. or i.p. or 20 μg of LPS. At 7-day intervals after the second injection, mice were bled and anti-thyroglobulin titers were determined in microtiter plates with SE coated (by the chromium chloride method) with thyroglobulin. Coating of SE with thyroglobulin was confirmed by reactivity with mouse serum which contained a known amount of anti-thyroglobulin (a generous gift from Yi-Chi M. Kong and N. R. Rose).

Plaque-forming cell assay. Four or five days after immunization of mice, the number of spleen cells producing antibody specific to the antigen used for immunizing was determined by a slide modification of the localized hemolysis-in-gel technique (10, 20). Depending on the immunizing antigen the indicator cells used to determine the number of specific PFC that were SE or SE coated with the appropriate antigen. In some experiments SE were coated with PVP by a tanning procedure (14) and used within 2 h after coating. PFC responses in mice sensitized to CGG or CGG-HE with or without *C. albicans* were assessed by using CGG-coated SE as described previously (18). Adult Leghorn chickens were sensitized and boosted twice by weekly i.v. injections of 2.0 ml of a 20% SE suspension in saline. Sera obtained 10 days after the last booster had an SE hemagglutination titer of 8,192.

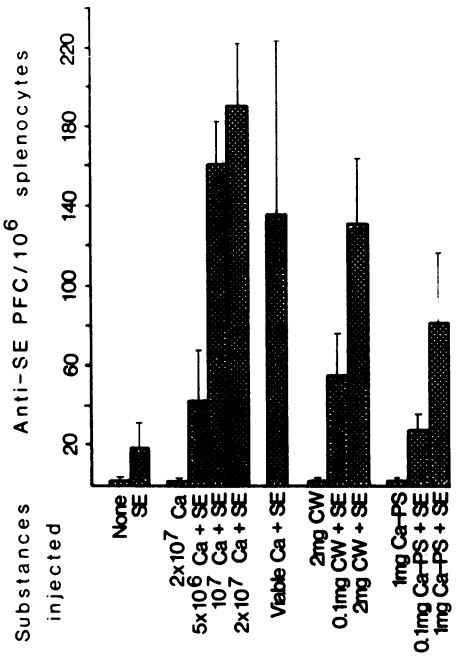

FIG. 1. Effect of *C. albicans* on immune response of BALB/c mice to SE. SE were given via an i.p. inoculation of 0.1 ml of 1% SE in saline. A dose of 0.1 ml of the appropriate concentration of heat-killed whole cells of *C. albicans* (Ca), cell walls (CW), or *C. albicans* polysaccharide (Ca-PS) was given i.p. at the time of inoculation of SE. One group of mice received 10^7 viable *C. albicans* i.p. 6 days before inoculation of 0.1 ml of 1% SE i.p. The cell wall preparation was from a glass bead breakage of whole cells as described previously (14). PFC were determined 4 days after inoculation of SE; bars indicate arithmetic means from four to six mice per group; lines indicate standard deviations.

SE were coated with CGG by adding an equal volume of 1% SE in saline to chicken anti-SE diluted just enough to prevent hemagglutination. Coating of SE with the CGG was confirmed by hemagglutination which occurred upon mixing the cells with goat anti-CGG.

Shellfish glycogen (type VI from oyster; Sigma) was used in some experiments as an inflammatory agent.

^{51}Cr labeling of SE. SE, washed three times with saline, were suspended in phosphate-buffered saline to a 10% suspension. Approximately 200 μCi of ^{51}Cr (as sodium chromate, NEZ-0305; New England Nuclear Corp.) was added per ml of the suspension, and the culture was gently mixed and incubated at 37°C for 1 h. The labeled cells were washed three times in phosphate-buffered saline and counted for specific activity. This procedure usually gave about 2×10^6 cpm/ml of a 1% suspension of erythrocytes. Although the cells were always used immediately after the labeling procedure, cells retained their label for several hours at room temperature.

RESULTS

Effect of *C. albicans* on immune response to SE. Mice injected i.p. with SE and dead yeast cells of *C. albicans* produced several times more splenic PFC against SE than did mice injected with SE alone (Fig. 1). Although similar results were obtained in BALB/c, DBA/1J, and Swiss-Webster mice, unless indicated otherwise the subsequent experiments were done on BALB/c animals. Also, immunopotentiation of comparable magnitude was induced by another strain of *C. albicans* (obtained from T. Mitchell, Duke University), three other species of *Candida*, and a strain of *Saccharomyces cerevisiae*. However, the remaining experiments were done on *C. albicans* strain 9938.

The immunopotentiation occurred when yeast cells were given at the time of injection of SE, but did not occur when injection of yeasts preceded the SE by 4 days. Mice preinjected with *C. albicans* still gave a heightened response to SE when a second injection of yeasts was given at the time of immunization with SE. Animals infected i.p. with viable yeasts 6 days before sensitization with SE also produced a heightened response to SE (Fig. 1). Microscopic examination of spleen cell preparations from infected animals revealed numerous yeasts. Animals given either dead or viable *C. albicans*, but no SE, did not have higher than background SE-specific PFC per spleen. The responses appeared to be specific because mice injected with *C. albicans* and SE did not have higher than background numbers of HE-specific PFC per spleen (data not shown).

Immunopotentiation was not dependent on whole cells of *C. albicans*. Cell wall fractions of yeast cells and *C. albicans* polysaccharide also enhanced mouse responses to SE (Fig. 1). An adjuvant effect occurred also in mice injected i.p. with 100 μg of *E. coli* LPS instead of *C. albicans* polysaccharide at the time of an i.p. injection of SE. From 2 to 4 times more SE-specific PFC per spleen were recovered from mice injected with LPS and SE than from mice injected with only SE.

C. albicans produced only a marginal effect when the fungus was given i.p. and the SE were given i.v. or when both materials were given i.v. (Table 1). It may be argued that *C. albicans*-induced immunopotentiation did not occur when 0.1 ml of a 1% suspension of SE was given i.v. because the response to SE was already at a physiological maximum (viz., 39,950 PFC per spleen). However, this was not the case because responses to an i.v. dose of SE 1/10 that of the above were likewise minimally affected by yeasts administered i.v. or i.p. (Table 1).

Effect of *C. albicans* on immune response to PVP and to CGG. Responses to PVP were not

TABLE 1. Effect of inoculation route on *C. albicans*-induced immunostimulation to SE

Dose of SE	Treatment	Anti-SE PFC[a]	
		Per spleen	Per 10^6 splenocytes
0.1 ml of a 1% suspension	SE i.p.	3,480 ± 4,640	15.6 ± 15.4
	SE i.v.	39,950 ± 18,850	146.8 ± 71.8
	SE i.p. + *C. albicans* i.p.	27,800 ± 14,927	99.6 ± 55.1
	SE i.p. + *C. albicans* i.v.	3,537 ± 2,707	13.3 ± 7.9
	SE i.v. + *C. albicans* i.v.	39,550 ± 21,753	116.0 ± 65.8
	SE i.v. + *C. albicans* i.p.	54,400 ± 38,581	211.1 ± 187.3
0.1 ml of a 0.1% suspension	SE i.v.	1,856 ± 1,066	10.5 ± 4.2
	SE i.v. + *C. albicans* i.v.	4,431 ± 4,153	16.7 ± 13.3
	SE i.v. + *C. albicans* i.p.	4,062 ± 1,873	19.3 ± 6.9
None	None	216 ± 152	1.63 ± 1.25

[a] PFC were determined 4 days after inoculation of SE; each value is expressed as the arithmetic mean per group ± standard deviation of mean from four to six mice per group. Similar results were obtained in repeat experiments.

statistically different in mice injected with PVP alone from mice injected with PVP and *C. albicans*. Responses ranged from 9,000 to 12,000 PFC per spleen when PVP alone or with the fungus was given i.v. and from 2,200 to 3,300 PFC per spleen when the antigen with or without *C. albicans* was given i.p.

A slight enhancement of response to CGG occurred when the protein was given i.p. with *C. albicans* (Fig. 2). However, when the CGG was coupled to HE, *C. albicans*-induced immunopotentiation of responses to CGG was much more remarkable.

Attempted induction of autoantibodies to thyroglobulin. An additional comparison was made between adjuvant activity of *C. albicans* and *E. coli* LPS by attempting to induce autoantibodies to thyroglobulin in DBA/1J (H-2^q haplotype) mice. By 21 days after the sensitizing dose of antigen, mice injected i.v. with mouse thyroglobulin and 20 μg of LPS developed an average anti-thyroglobulin passive hemagglutination $\log_2$ titer of 10 (range, 7 through 13). Mice injected with thyroglobulin alone had an average titer of 2.4 (range, 1 through 4); mice injected i.v. with thyroglobulin and 500 μg of *C. albicans* polysaccharide averaged 4.6 (range, 1 through 6); mice injected i.v. with thyroglobulin and 100 g of *C. albicans* polysaccharide averaged 2.8 (range, 1 through 6); and mice injected i.p. with thyroglobulin and 10^7 *C. albicans* cells averaged 2.6 (range, 1 through 5). These data indicate that whereas LPS induces a high titer of autoantibodies to mouse thyroglobulin, the effects of *C. albicans* on this induction are minimal.

Effect of *C. albicans* on tissue distribution of SE. The poor potentiation or adjuvant activity of *C. albicans* when soluble antigens are used, when the fungus is given i.v., or when particulate antigen is given i.v. instead of i.p. may suggest that the immunopotentiation is not due to activation of cells such as macrophages or lymphocytes. Rather, coinjection of a particulate antigen and *C. albicans* at a local site may somehow influence the rate of presentation of antigen to the immune system. To test this idea

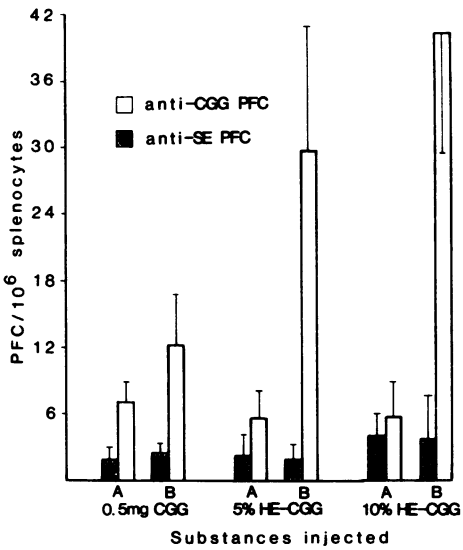

FIG. 2. Effect of *C. albicans* on immune response to free CGG and to CGG attached to HE (HE-CGG). Mice were inoculated i.p. with 0.1 ml of the appropriate antigen preparation either alone (A) or in combination with 10^7 killed *C. albicans* yeast cells (B). PFC specific for CGG and for SE (as controls) were determined 4 days after inoculation of antigen; bars indicate arithmetic means from four to six mice per group; lines indicate standard deviations.

TABLE 2. Effect of *C. albicans* on tissue distribution of SE

Time (h) post-inoculation	Route of inoculation	Material injected[a]	cpm/g of tissue[b]			
			Inguinal lymph node	Mesenteric lymph node	Spleen	Liver
2	i.p.	^{51}Cr-SE	NS[c]	30,279 ± 5,142	51,155 ± 3,049	15,344 ± 5,101
		^{51}Cr-SE + *C. albicans*	NS	40,396 ± 29,596	57,857 ± 6,095	56,123 ± 8,582
6	i.p.	^{51}Cr-SE	NS	62,689 ± 10,521	64,501 ± 37,049	46,488 ± 16,924
		^{51}Cr-SE + *C. albicans*	4,517 ± 1,754	48,852 ± 49,128	111,969 ± 11,636	154,960 ± 44,711
2	i.v.	^{51}Cr-SE	NS	NS	54,682 ± 24,986	221,394 ± 57,280

[a] Doses of 0.1 ml of a 1% suspension of ^{51}Cr-labeled SE (approximately 3 × 10^6 cpm/ml) were inoculated i.p. In some cases the inoculum also contained 10^7 killed *C. albicans* yeasts.

[b] At appropriate times post-inoculation mice were exsanguinated, and the indicated tissues were removed, rinsed several times in saline, blotted, weighed, and placed in vials for determination of counts per minute in a gamma counter. The numbers expressed represent means and standard deviations from an experiment involving four mice per group. Repeat experiments gave similar results.

[c] NS, Not significantly greater than background.

^{51}Cr-labeled SE were injected i.p. alone or with *C. albicans*. At different times after the injection the distribution of SE in inguinal lymph nodes, mesenteric lymph nodes, spleen, and liver was determined. By 2 h postinjection the appearance of radiolabel in the lymph nodes and spleen was relatively unaffected by the presence of yeasts in the peritoneal cavity. The liver, however, contained more radiolabel when yeasts were injected i.p. along with the labeled SE (Table 2). By 6 h the amount of radiolabel appearing in the liver approached the amount of radiolabel trapped by the liver in 2 h after an i.v. injection of labeled SE. It is unlikely that the increase in counts associated with the liver is due to nonspecific adsorption of labeled SE on the organ surface because (i) all organs were vigorously rinsed twice in saline before counting, and (ii) a *C. albicans*-associated increase in radiolabel of other peritoneal organs did not occur.

Because *C. albicans* activates the alternative complement cascade (21, 26), enhancement of liver uptake of particulate antigen in the peritoneal cavity may be associated with induction of acute inflammation by the fungus. To test this idea, shellfish glycogen was used to determine its effect on liver uptake of ^{51}Cr-labeled SE. By 2 h postinjection, animals which received a combination of labeled SE and glycogen had 3 to 4 times more radiolabel in their liver than did animals which received only SE (data not shown).

An association of inflammation (and rapid uptake of antigen by the liver) with enhanced immune responsiveness was further demonstrated by examining the effect of glycogen on immune responsiveness of mice to SE. Mice injected i.p. with SE and glycogen developed more SE-specific PFC per spleen than did mice injected i.p. with SE alone (Table 3).

It may be expected that products of an acute inflammatory response will lead to lysis of SE. Such lysis, however, is either not important or not in itself sufficient to explain the immunopotentiation because mice responded poorly to an i.p. injection of lysed SE (Table 3).

DISCUSSION

We demonstrated that *C. albicans* can induce an enhanced response in inbred and outbred strains of mice to antigens unrelated to the yeast. Conditions which favor this event include the use of a particulate antigen, injection of antigen and fungus simultaneously, and injection of both substances i.p.

C. albicans cell walls and soluble extracts,

TABLE 3. Effect of glycogen on immune response to SE and immune response to lysed versus intact SE in BALB/c mice

Treatment of mice[a]	Anti-SE PFC[b]	
	Per spleen	Per 10^6 splenocytes
SE	3,406 ± 1,174	20.5 ± 8.6
Glycogen	450 ± 147	2.4 ± 0.8
SE + glycogen	45,937 ± 23,878	220.4 ± 75.8
Lysed SE	268 ± 132	2.3 ± 1.1

[a] Mice received either 0.1 ml of a 1% suspension of SE or 0.1 ml of a 5% solution of glycogen in saline i.p., or they received both materials i.p. One group of mice received i.p. 0.1 ml of lysed SE which were prepared by taking 1 ml of a 1% suspension of SE, pelleting the cells by centrifugation, suspending in 1 ml of distilled water, and then bringing the solution to 0.15 M saline by appropriate addition of sterile NaCl.

[b] PFC were determined 4 days after inoculation; each value is expressed as the arithmetic mean per group ± standard deviation of mean from four mice per group.

including the *C. albicans* polysaccharide used in these studies, possess endotoxin-like activities (3). Comparing adjuvanticity of the fungus with bacterial endotoxin (LPS) we found that although immunopotentiation of anti-SE responses occurred after an i.p. injection of SE with either adjuvant, mechanisms of adjuvanticity by the fungus appeared more limited than by the bacterial LPS. LPS may suppress immune responses when given several days before antigen (5, 11), but injection of *C. albicans* 4 days before antigen did not affect subsequent immune responses to the antigen. Whereas LPS induced high titers of anti-thyroglobulin when the adjuvant was administered to mice along with mouse thyroglobulin as previously described (8), neither whole cells nor the polysaccharide extract of *C. albicans* produced a significant effect. These findings provide evidence, in addition to that cited previously (3), of differences in biological activity between bacterial endotoxin and *C. albicans*.

Several lines of evidence indicate that induction of an inflammatory reaction is a critical event for a *C. albicans*-induced immunopotentiation to occur. As stated above, the fungus should be given not only at the same time as antigen, but also in the same site. Adjuvanticity is not restricted to *C. albicans*, but occurs when other species of *Candida* and *S. cerevisiae* are used—all species of which should be expected to activate the alternative complement cascade (19, 21, 26) and will induce an acute inflammatory response when injected i.p. (our unpublished observations). Another inflammatory agent, shellfish glycogen (33), also induced enhanced responses against SE when the irritant was injected i.p. at the time of injection of SE by the same route.

Induction of inflammation in the peritoneal cavity correlated with a more rapid uptake by the liver of SE from the cavity (Table 2). Several observations suggest that this correlation may be important. First, glycogen, which promoted inflammation and caused enhanced anti-SE responses, also promoted a more rapid uptake by the liver of SE from the peritoneal cavity (our unpublished data). Second, response to the protein antigen CGG was only marginally affected by *C. albicans* (Fig. 2), but when CGG was presented as attached to HE the response was greatly enhanced by the yeast. It may be expected that free antigen given i.p. would be rapidly taken up by the liver in the absence of inflammation, but when attached to HE vascular absorption of antigen from the peritoneal cavity occurs more slowly. In support of this idea we found that, after an i.p. injection of free ^{51}Cr, liver uptake of the radionuclide occurred just as rapidly with or without *C. albicans* (unpublished data). Third, the effect of *C. albicans* on anti-SE responses was marginal when SE were administered i.v. Actually the magnitude of the immune response to an i.p. injection of a 1% suspension of SE and *C. albicans* approached that of an i.v. injection of SE alone (Table 1).

The mechanism by which antigen trapping by the liver may be translated into an immune response is unknown. Although it may be assumed that in our studies SE are taken up by Kupffer cells, the role of these macrophages in antigen-specific immune responses is controversial (23, 30). Alternatively the increased rate of liver uptake may be a coincidental event, and the important effect of peritoneal inflammation is induction of some other activity such as alteration of lymphocyte trafficking (9).

The effect of yeasts on enhancing resistance to diseases such as tumors and infectious agents may involve mechanisms either in addition to or other than those proposed here for enhanced antibody responses. Yeast cell walls and water-insoluble polysaccharides have been shown to inhibit certain types of tumors in mice and rats (1, 6, 17, 31), and a water-insoluble glucan from *S. cerevisiae* increases resistance in animals to certain bacterial and viral diseases (28). In the latter study, resistance in some animals correlated with a glucan-induced increase in antibody titers to the infectious agent. However, the polysaccharide was effective when administered i.v. and before injection of the infectious agents.

In our studies it is interesting that animals infected with live yeasts several days before giving SE developed enhanced anti-SE responses (Fig. 1). If *C. albicans* is capable of inducing heightened antibody responses to cell-associated antigens in general, it may be logical to ask whether animals infected with this fungus tend to develop antibodies against their own cellular antigens. Autoantibodies and endocrinopathies of one or more organs have been found associated with certain forms of human candidiasis such as chronic mucocutaneous candidiasis (7, 26). In many instances it is not clear whether autoantibody formation and endocrine disorders precede candidiasis or develop as a consequence of the fungal disease. Although many workers who investigate fungus-host interactions have concentrated on the effect of the host on the fungal agent, it is clear that further work is needed in understanding how the fungus affects the host.

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