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Synergistic Effect of *Candida albicans* and *Staphylococcus aureus* on Mouse Mortality

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A synergistic effect on mouse mortality was demonstrated in combined infection of mice with *Candida albicans* and a *Staphylococcus aureus* strain isolated from a patient with toxic shock syndrome. Mice exhibited high resistance when inoculated intraperitoneally by either pathogen alone. Dual infection with the two organisms together, however, at doses which separately caused no animal deaths, resulted in 100% mortality. This synergistic effect could not be reproduced when either of the agents was heat inactivated.

Candidosis is well known for its occurrence with a variety of diseases (3, 8, 23, 28). The cause-effect role of *Candida albicans* in these many conditions remains unclear and is considered to be anything from a cause of a minor accompanying infection (7) to a major contributing factor in serious illnesses (10, 16, 17, 26). Literature describing clinical candidosis states that *C. albicans* is frequently found with *Staphylococcus aureus* and *Streptococcus* spp. (13, 16), and in vitro *C. albicans* have been found to enhance the growth of a number of bacteria, including *S. aureus* (27).

Currently, candidosis is widespread with more than 50% of the population exhibiting a positive delayed hypersensitivity response (22). It is a common cause of vaginitis (12), and in many women it is reported to occur premenstrually and repeatedly (6, 11, 25). Even in normal women, asymptomatic vaginal carriage is high. *Candida albicans* was isolated from 11.3% of women studied, according to one report (14). Recently, reports described *Staphylococcus* spp. infections also occurring at menses with a greater than normal risk of reoccurrence (5).

The purpose of this study is to investigate the possibility of a synergistic relationship between these two organisms in the experimental situation.

MATERIALS AND METHODS

Mice. Inbred mice of the strain CD-1 were obtained from Charles River Laboratories, Wilmington, Mass. Male mice weighing between 22 and 25 g were used.

Pathogens. *S. aureus* 2460 was received from the Michigan Department of Public Health, which originally obtained it from an Ann Arbor Hospital where it was isolated from a patient with confirmed toxic shock syndrome according to the criteria of the Centers for Disease Control (4). It has the phage group I sensitivity pattern (24). The *C. albicans* used was from the microbiology laboratory shock culture collection at Michigan Technological University. Cultures used for injection were grown on 5% sheep blood agar (blood agar base [BBL Microbiology Systems] plus whole blood) for 24 h at 37°C (*S. aureus*) or Sabouraud dextrose agar (BBL) for 48 h (*C. albicans*), eluted and washed with sterile physiological saline, mixed vigorously, and used immediately. Heat inactivation was achieved by boiling 10-ml saline suspensions at concentrations to be used in injections for 10 min. Titers were estimated at the time of infection from a calibration curve relating the optical density at 660 nm (Coleman Junior spectrophotometer) to colony-forming units (CFUs). CFUs were determined by plate counts on blood and Sabouraud agar media.

Injections. Organisms were injected intraperitoneally with the desired dose suspended in 0.2 ml of saline. When only one agent was used, 0.2 ml saline was substituted for the second agent. Injected animals were observed every 12 h for death or symptoms of disease. Animals were closely observed for 1 week after injection. Where death was anticipated, animals were watched very closely, and autopsies were conducted immediately after death. Representative autopsies were done on animals which died during the daytime hours.

Animal tissues. Tissues were sterilely removed from infected animals immediately after death. The entire organs (or 0.5 g of intestine) were rinsed quickly in 70% alcohol to remove any surface contamination, rinsed in sterile saline, and homogenized with a mortar and pestle; nutrient broth was used to make a final volume of 1 ml.

The surfaces of the peritoneal cavity were wiped with a moist Q-tip swab which was placed in the 1 ml of broth. CFU determinations were made on these 1-ml organ samples.

The pathogen content was determined by vigorous mixing and dilution in nutrient broth. Samples were then placed in duplicate on selective media. CFUs of *S. aureus* were determined by using *Staphylococcus* 110 agar medium (Difco), and *C. albicans* CFUs were

TABLE 1. LD_{50} for *S. aureus* and *C. albicans*

S. aureus CFU	*C. albicans* CFU	Dead mice/total
	1×10^6	0/6
	7×10^7	1/6
	2.4×10^8	1/6
	2.9×10^8	5/10
	6.0×10^8	6/6
1.4×10^9		0/6
3.0×10^9		0/6
5.5×10^9		5/8
9.0×10^9		5/6
5.3×10^9[a]	2.9×10^8[b]	

[a] LD_{50}; results were calculated according to the moving average method with 95% confidence limits between 3.8×10^9 and 7.5×10^9 (1).

[b] LD_{50}; 95% confidence limits between 2.2×10^8 and 3.9×10^8.

allowed to develop on Sabouraud dextrose agar containing 100 mg of chloramphenicol (Sigma Chemical Co.) per liter.

RESULTS

The doses causing 50% mortality in 7 days (LD_{50}) for *C. albicans* and *S. aureus* 2460 for mice injected separately and intraperitoneally are given in Table 1. A dose of 5.3×10^9 CFUs of *S. aureus* or 2.9×10^8 CFUs of *C. albicans* was required.

The effect on mouse mortality of combined doses is presented in Table 2. It is observed that doses causing no mortality alone caused high mortality in combination. Heat inactivation eliminated this synergistic effect. In a number of separate experiments it appeared that to get this effect each organism must be present in a certain minimal amount with the ideal ratio being 10:1 *S. aureus* to *C. albicans*. In experiment 1, where the *S. aureus* dose was 8.0×10^8 CFU and the *C. albicans* dose was 7.0×10^7 CFU, 83% of the animals died on the second day within a 24-h time period (Fig. 1).

Animals receiving combined doses showed symptoms of bilateral conjunctivitis, dark noses, and inactivity. Upon autopsy the most obvious pathological observation was a greatly enlarged, gold-colored small intestine. These symptoms were not observed in the animals receiving one agent alone at doses used in the combined infection. Table 3 illustrates relative numbers of CFUs in sampled organs. It can be seen that infection was widespread and all abdominal organs sampled were infected. In dually infected animals *S. aureus* predominated, but both organisms were present at levels similar to that found in animals killed by the LD_{50} of each pathogen alone.

DISCUSSION

Murine cytomegalovirus (9), cortisone (15), and X-rays (19) have been shown to reduce the resistance of mice to experimental infection with *C. albicans*.

Sporadic early papers reported often contradictory and inconclusive results of studies carried out on animals simultaneously inoculated with *C. albicans* (or extracts of *C. albicans*) and various bacteria (or extracts of bacteria). For a review of these reports see reference 18. Studies on synergistic effects of *C. albicans* and other microbes have not been actively pursued.

TABLE 2. Effect of combined doses of *S. aureus* and *C. albicans* on mortality in mice

Expt	Treatment group	Dose of *C. albicans* (CFU)	Dose of *S. aureus* (CFU)	Dead mice/total
1	A_1	7.0×10^7	8.0×10^8	6/6
	B_1		8.0×10^8	0/6
	C_1	7.0×10^7		1/6[b]
2	A_2	1.0×10^8	1.4×10^9	6/6
	B_2		1.4×10^9	0/6
	C_2	1.0×10^8		0/6
3	A_3	1.7×10^8	9.0×10^8	10/13
	B_3		9.0×10^8	0/6
	C_3	1.7×10^8		0/6
4	A_4	1.9×10^8	1.4×10^9	5/6
	B_4	1.9×10^8 HI[a]	1.4×10^9	0/6
	C_4	1.9×10^8	1.4×10^9 HI[a]	0/6
5	A_5	1.3×10^8	2.5×10^8	0/6
	B_5	4.0×10^7	1.5×10^9	0/6

[a] HI, Heat inactivated.

[b] Autopsy of dead animal revealed extensive bacterial infection.

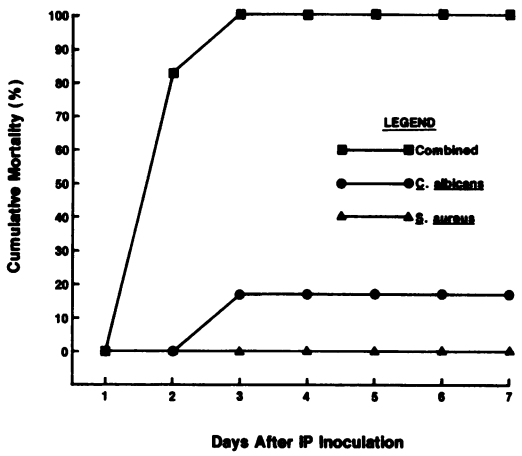

FIG. 1. Cumulative mortality in mice of experiment 1. Mice were infected intraperitoneally with 8.0 $\times 10^8$ CFUs of *S. aureus* or 7.0 $\times 10^7$ CFUs of *C. albicans* or both.

In the experiments reported here it appears that *C. albicans* and *S. aureus* interact synergistically to cause high mortality in mice. In one experiment *C. albicans* at 1/4 of its LD_{50} combined with *S. aureus* at 1/8 of its LD_{50} resulted in 100% mortality in exposed animals.

Upon autopsy of the animals killed by the combined inoculations, infections were revealed in all abdominal organs examined with *S. aureus* predominating over *C. albicans* by a ratio of around 100:1. However, the CFU numbers of *S. aureus* were also higher than those of *C. albicans* in tissues of animals killed by an LD_{50} of each organism alone, so it was not possible to say that one organism was mainly responsible for death in the dual infection.

It must be noted that vaginal isolates of *S. aureus* from toxic shock syndrome patients have been distinguished for their lack of invasiveness (attributed to their failure to produce high concentrations of hemolysin, lipase, and nuclease) while elaborating large amounts of pyogenic exotoxin C (21).

The expression of exocellular products has not been studied for the *S. aureus* strain used here. However, work is currently in progress to determine whether only certain or all strains of *S. aureus* act synergistically with *C. albicans* in the manner described in this report. So far *S. aureus* isolates from toxic shock syndrome-associated vaginal, wound, infant scarlet rash, and non-disease-associated sources all act similarly when combined with *C. albicans* with regard to invasiveness and mouse mortality, but differences in symptomatology are observed (manuscript in preparation).

It must be emphasized that the study reported here was not designed as a model for human disease but rather to determine first whether a synergistic effect on a disease process existed between these two pathogens. The experimental conditions chosen were designed to optimize such an effect, and nothing can be said about how these organisms would behave under a natural disease situations. It is noteworthy that *C. albicans* was also invasive in this experimental situation, but the vast majority of cases of human candidosis are superficial in nature and limited to the alimentary and vaginal surfaces (6, 12, 26).

As a second step in determining the possibility of actual synergistic role of these two organisms an animal model more closely resembling a human situation could involve the establishment of chronic animal candidosis of the alimentary tract (20) followed by exposure to *S. aureus* strains by various routes of inoculation.

C. albicans has long been recognized for its ability to invade the immunologically weakened host (18, 28). Presently many chemicals are being identified which weaken the immune system (for review, see reference 2, and clinical reports indicate that candidosis is widespread in the human population (22, 26).

TABLE 3. Recovery of *C. albicans* and *S. aureus* from mice killed by dual and single inoculations

Infection type	Organ	CFUs per organ sample[a]	
		C. albicans	*S. aureus*
Dual injection[b]	Peritoneum	2×10^5	4×10^7
	Large intestine	1×10^5	1×10^7
	Small intestine	1×10^4	5×10^6
	Spleen	1×10	1×10^7
	Pancreas	2×10^4	2×10^7
Single LD_{50} injection[c]	Peritoneum	1×10^6	3×10^7
	Large intestine	1×10^5	1×10^5
	Small intestine	7×10^5	5×10^6
	Spleen	5×10^3	2×10^5
	Pancreas	6×10^2	5×10^4

[a] No *S. aureus* or *C. albicans* CFUs were found in uninfected animals indicating less than 100 per sample. For titrations the entire organ was homogenized in 1 ml of broth, except for the intestine, where 0.5 g was used, and the peritoneum, where surfaces were wiped with a wet swab and the swab was placed in 1 ml of broth. (Titrations were also done on kidneys. Great variations in the degree of infection were observed. In some cases no CFUs were detected in one kidney, whereas the other kidney from the same animal contained lesions yielding great numbers of organisms.)

[b] Data represent averages from three animals infected with 1.2×10^8 CFUs of *C. albicans* and 1.0×10^9 CFUs of *S. aureus*.

[c] Data represent averages from three animals infected with 2.9×10^8 CFUs of *C. albicans* or 5.5×10^9 CFUs of *S. aureus*.

In recent years a number of new serious diseases have been reported involving organisms carried by many without clinical symptoms (5, 8). As *C. albicans* is so frequently present, the possibility exists that it may be playing a much more important role in disease than previously suspected. Indeed, the recent clinical reports relating candidosis to a variety of disease symptoms support this idea and call for a new appraisal of the role of *C. albicans* in disease (25, 26).

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