PRODUCTION OF TOLERANCE TO THE TOXICITY OF Candida Albicans BY NONFUNGAL MATERIALS

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ABSTRACT

Hasenclever, H. F. (National Institute of Allergy and Infectious Diseases, Bethesda, Md.) and W. O. Mitchell. Production of tolerance to the toxicity of Candida albicans by nonfungal materials. J. Bacteriol. 84:1325–1329. 1962.—Tolerance in mice to the toxic manifestations of viable Candida albicans cells was elicited by injections of Salmonella enteritidis or S. typhosa lipopolysaccharides, or complete Freund’s adjuvant. The greatest host stimulation occurred when the lipopolysaccharides were given 1 or 6 days before intravenous challenge. The highest level of tolerance produced by intraperitoneal preinfection with C. albicans was found when the yeast cells were injected 6 days before challenge. Older mice appeared to require larger amounts of endotoxin to demonstrate toxicity tolerance. Protection in mice that received both lipopolysaccharide injections and C. albicans intraperitoneal preinfection was slightly higher than in those mice given only the individual components.

The lethal toxicity of whole viable Candida albicans yeast cells for normal mice has been reported by several investigators (Mourad and Friedman, 1961; Hasenclever and Mitchell, 1962a). Recently, Hasenclever and Mitchell (1962a, b) reported that mice could be made tolerant to the acute toxic manifestations of C. albicans by sublethal infections with antigenically related or unrelated pathogenic fungi, Salmonella lipopolysaccharides, or complete Freund’s adjuvant. Although the majority of the treated mice survived the acute toxic reaction observed after intravenous challenge with large numbers of this yeast, they eventually succumbed to an overwhelming infection. The average survival time (ast) of the tolerant mice was three to six times that of the untreated controls. Little differences in the in vivo growth rates of C. albicans between treated and control mice could be detected up to 24 hr postchallenge, the time at which most or all of the control mice were dead.

This paper reports in detail the production of tolerance to the acute toxicity of C. albicans by Freund’s adjuvant and Salmonella lipopolysaccharides, singly and in combination with other tolerance-inducing agents.

MATERIALS AND METHODS

The animals used in this study were Swiss white mice, and ranged in age from 6 to 12 weeks. Both males and females were used, but females were utilized for the majority of experiments.

Freund’s adjuvant and S. enteritidis and S. typhosa lipopolysaccharides were purchased from Difco Laboratories.

The lipopolysaccharides were dissolved at the desired concentration in sterile distilled water or sterile physiological saline, and were injected intraperitoneally. The Freund’s adjuvant was also administered ip: 1 mg (dry wt) of Mycobacterium butyricum per 0.1 ml of oil; 0.1 ml of the complete adjuvant was given to each mouse.

The strain of C. albicans used for this study was B311. Yeast cells for intravenous challenge were harvested from stationary 2% glucose, 1% neopeptone broth cultures grown for 48 to 72 hr at 30 C. For toxicity studies, mice were injected intravenously with $10^7$ to $2 \times 10^7$ yeast cells. Yeast-cell populations were determined by direct count in a Levy hemocytometer, and by quantitative plating procedures.

After intravenous challenge, the mice were observed for deaths at 1- to 3-hr intervals up to 12 hr postchallenge, and subsequently at 3- to 8-hr intervals to the end of the experiment. The percent cumulative death curves and ast in hours,
for each experimental group, were calculated from the observed mortality rates.

Coccidioides immitis spherules were obtained from George Lones. Viability was destroyed by exposure to 1% formaldehyde for 24 hr at 30 C. The spherules were washed several times and passed through a French pressure cell at 25,000 psi. This resulted in complete rupture of all cellular entities. The fragments were collected by centrifugation, and the equivalent of 1 mg (dry wt) was injected per mouse.

RESULTS AND DISCUSSION

The extension of the ast in mice receiving C. immitis spherule fragments or Salmonella lipopolysaccharides is shown in Fig. 1. The groups of animals treated with these materials survived three to six times longer than the controls, or those that received sterile distilled water. The two

![Graph showing production of tolerance by several different agents.](http://jb.asm.org/)

**FIG. 1.** Production of tolerance by several different agents. Group 1: 1.0 mg of Coccidioides immitis spherule fragments ip 6 days before challenge, 29 mice; group 2: 0.1 ml of distilled water ip, room temperature, 5, 3, and 1 days before challenge, 30 mice; group 3: 0.1 ml of distilled water ip, 70 C, 5, 3, and 1 days before challenge, 30 mice; group 4: Salmonella enteritidis lipopolysaccharide, 20 μg ip 6 days before, and 30 μg ip 1 day before challenge, 30 mice; group 5: S. typhosa lipopolysaccharide ip, 20 μg 6 days before and 30 μg 1 day before challenge, 30 mice; group 6: controls, 70 mice.

latter groups were included as controls for the lipopolysaccharide-treated mice, since these substances were dissolved in distilled water. It is quite apparent that the amount of water given had little or no effect.

Figure 2 shows the effect of Freund's adjuvant, the component parts of this adjuvant, and S. enteritidis lipopolysaccharide, given at different times and amounts, upon altering the response in treated and untreated mice to the toxicity of C. albicans. The individual Freund's ingredients, when given separately, produced little protective response, but, when combined, an extended ast was observed. When 30 μg of S. enteritidis lipopolysaccharide were given 1 or 6 days before challenge, a beneficial effect was seen; 5 μg in-
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Fig. 3. Group 1: 30 µg of Salmonella enteritidis lipopolysaccharide ip 14 days before challenge, 29 mice; group 2: 10⁷ viable Candida albicans cells ip 14 days before challenge, 28 mice; group 3: 30 µg of S. enteritidis lipopolysaccharide ip 10 days before challenge, 28 mice; group 4: 10⁷ viable C. albicans cells ip 10 days before challenge, 30 mice; group 5: 30 µg of S. enteritidis lipopolysaccharide ip 6 days before challenge, 30 mice; group 6: 10⁷ viable C. albicans cells ip 6 days before challenge, 29 mice; group 7: controls, 40 mice.

Fig. 4. Group 1 (group 5 of Fig. 3): 30 µg of Salmonella enteritidis lipopolysaccharide ip 6 days before challenge, 30 mice; group 2 (group 6 of Fig. 3): 10⁷ viable Candida albicans cells ip 6 days before challenge, 29 mice; group 3: 30 µg of S. enteritidis lipopolysaccharide ip 3 days before challenge, 30 mice; group 4: 10⁷ viable C. albicans cells ip 3 days before challenge, 30 mice; group 5: 30 µg of S. enteritidis lipopolysaccharide ip 1 day before challenge, 30 mice; group 6: 10⁷ viable C. albicans cells ip 1 day before challenge, 30 mice; group 7 (group 7 of Fig. 3): controls, 40 mice.

Data presented in Fig. 1 and 2 indicate that tolerance to the acute toxicity of C. albicans, stimulated by S. enteritidis lipopolysaccharide, was apparent when this agent was injected 1 or 6 days before challenge. Earlier studies with intraperitoneal preinfection with C. albicans (Hasenclever and Mitchell, 1962b) showed that when the sublethal infection was given 6 days before challenge, tolerance was observed in the recipient mice. Figures 3 and 4 show the protection produced by the injections of lipopolysaccharide or viable C. albicans at varying times before challenge. Under the conditions of this experiment, the lipopolysaccharide elicited its greatest protective effect when given at 1 or 6 days before challenge. When the endotoxin was injected 3 days before challenge, less protection was observed than when given 1 day before challenge. This bimodal manifestation of the tolerant state has been observed repeatedly. The maximal protection afforded by the intraperitoneal preinfection was seen when the yeast cells were given 6 days of prechallenge.

During the period of experimentation, we noted that older mice (7 to 10 weeks) appeared to be more refractile to stimulation by some of the tolerance-producing agents than were younger mice. Figure 5 shows the effect of S. typhosa lipopolysaccharide in producing tolerance to the toxicity of C. albicans in 12-, 9-, and 6-week-old mice. Under the conditions of this experiment, 12- and 6-week-old mice responded to the endotoxin, but the 9-week-old mice failed to respond.

Figure 6 shows the effect of multiple injections of lipopolysaccharide or viable C. albicans cells...
in producing tolerance to the toxicity of this yeast in 9-week-old mice. The ast of the endotoxin-treated group (Fig. 6) was eight times that of the controls. Two injections of viable C. albicans cells failed to produce any increased resistance. It appears that more lipopolysaccharide is required to produce tolerance in older and larger mice. When comparing the results of this experiment with those presented in Fig. 5, it must be noted that S. typhosa lipopolysaccharide was used in the former, whereas S. enteritidis lipopolysaccharide was utilized for this experiment. According to the results shown in Fig. 1, however, little difference in response to the two lipopolysaccharides was noted.

Figure 7 shows the tolerance to C. albicans toxicity of mice that received injections of both lipopolysaccharide and viable C. albicans cells. The extended survival of mice administered both agents individually, but on the same day, was somewhat greater than the groups that received only one agent. A similar observation was noted in the mice of group 5, which were injected intraperitoneally with viable C. albicans cells 6 days before, and lipopolysaccharide 1 day before, challenge. The additive protection conferred to groups 3 and 5 was slight, however.

In an earlier report, we (1962b) showed that mice made tolerant to the toxicity of C. albicans with various fungal preparations demonstrated a slight neutrophilic response. To test that observation more completely, total and differential counts were done with blood from mice that had received multiple injections of lipopolysaccharide or one injection each of lipopolysaccharide and 1.5 X 10⁷ viable C. albicans cells ip. These methods (Fig. 6 and 7) indicate the greatest stimulation of tolerance in the recipient mice. The neutrophilic response, however, was no greater than that already reported (Hasenclever and Mitchell, 1962b).

Although the acquired tolerance of mice to the toxins of C. albicans may appear to be non-specific in nature, one common relationship exists. The protection is conferred by injections of bac-
FIG. 7. Group 1: 10^7 viable B311 cells ip 6 days before challenge, 30 mice; group 2: 30 μg of Salmonella enteritidis lipopolysaccharide ip 6 days before challenge, 29 mice; group 3: 10^7 viable B311 cells and 30 μg of S. enteritidis lipopolysaccharide ip 6 days before challenge, 28 mice; group 4: 30 μg of S. enteritidis lipopolysaccharide ip 1 day before challenge, 29 mice; group 5: 10^7 viable B311 cells ip 6 days, and 30 μg of S. enteritidis lipopolysaccharide 1 day before challenge, 28 mice; group 6: controls, 40 mice.

In contrast to nonviable C. albicans cells, this preparation was quite effective in stimulating tolerance to the toxins of C. albicans.

Cross protection in mice against a wide variety of microorganisms, after injection with crude or partially purified lipopolysaccharides from members of Enterobacteriaceae and some other gram-negative bacilli, has been reported (Field, Howard, and Whitby, 1955; Landy, 1956; Dubos and Schaedler, 1956). S. enteritidis and S. typhosa lipopolysaccharides were quite effective stimulants of the tolerance described here. These observations reemphasize that the host response is such that it is capable of coping with a wide variety of pathogenic microorganisms. We have not eliminated the possibility that common endotoxins exist between the fungi and Salmonella studied. The evidence presented here, however, does not favor that hypothesis.

Although these studies revealed some interesting results, they raised more questions than they answered. There is no doubt that protection to the acute toxicity of C. albicans can be induced by the procedures described; however, the basic host mechanism and its relationship with resistance to chronic candidiasis remain unknown.

LITERATURE CITED


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