TOXICITY OF BLASTOMYCES DERMATITIDIS

by

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ABSTRACT

Following injections of the insoluble portion of disrupted yeast cells of Blastomyces dermatitidis animals exhibited a biphasic pyrogenic response, a decrease in total serum proteins, and there was no release of interferon.

INTRODUCTION

The implication of fungal toxins as health hazards has stimulated considerable research dealing with various aspects of their biologic activity. Aflatoxins were implicated in the poisoning of farm animals (Wogan, 1966) and were shown to be carcinogens (Wogan, 1966). Candida endotoxin contributes to the pathogenesis of this organism (Seelig, 1966). Cellular preparations and protoplastic preparations of Blastomyces dermatitidis were found to be lethal for mice when injected concurrently with BCG (Salvin, 1952), and trypsin treatment or mild HCl treatment of yeast cells of this fungus was shown to enhance the lethality (Taylor, 1964). However, BCG was again used as an adjuvant. It was also demonstrated that B. dermatitidis endotoxin was present in a particulate suspension prepared from disrupted yeast cells and prior injection of Newcastle disease virus (NDV) in mice enhanced the lethality (Huang & Landay, 1969). This report contains further observations on the responses to suspensions of B. dermatitidis in animals.

MATERIALS AND METHODS

French press suspension

Yeast cells of B. dermatitidis (Tonar isolate) 3) were grown on peptone glucose agar, suspended in merthiolated saline 1/10,000 v/v, washed three times by centrifugation in an International centrifuge

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Accepted for publication: 17.II.1971
and killed with acetone. After the acetone was removed by centrifugation and evaporation under vacuum the cells were disrupted in a french press until microscopic evidence of at least 70% breakage was observed. The sediment was then recovered and washed three times in merthiolated saline in the Servall refrigerated centrifuge at 28,000 x g. After the sediment was suspended in this diluent, a dry weight determination was made and the suspension was tested for sterility in Sabouraud’s broth.

**Pyrogenic response**

Normal rabbits (miniature New Zealand, 4-6 lbs) were immobilized in a wooden box and temperatures were measured with a rectal thermometer until two consecutive readings were unchanged. Three animals were then injected (IV) with 22.5 mg (in 0.25 ml) of suspension and three animals with 0.25 ml of merthiolated saline. Temperatures were taken periodically.

**Serum protein electrophoresis**

Changes in the total serum protein of the miniature 4-6 lb white New Zealand rabbits was assayed by refraction following an I.P. injection of 1 ml (70 mg or 35 mg) of the french press suspension. Two control animals received 1 ml of merthiolated saline and 70 mg of chitin (practical grade, MATHESON, COLEMAN and BELL), respectively.

Sera from several of the rabbits were separated on cellulose polyacetate electrophoresis strips (Sepaphore III, Gelman Instrument Co.) placed in a Gelman electrophoretic chamber with the voltage set at 250 for 35 minutes. Tracings and quantiation of the albumin and globulin fractions were then made in a chromoscan (Joyce Loebl & Co., Ltd., Gateshead on Tyne II).

**Interferon**

Mice were divided into four groups, the first of which was injected (I.P.) with 1 ml (1,000 μg) of *Escherichia coli* lipopolysaccaride W (026:B6; Difco) dissolved in physiologic saline. The second group was injected (I.P.) with 1 ml (50 mg) of the *B. dermatitidis* french press suspension. Two control groups received I.P. injections of 1 ml of the respective dilutents. Six mice from each group were bled by decapitation at 0, 1.5, 3 and 6 hours post injection. The sera obtained from each group at each time interval were pooled and frozen at -35°C.

After storage these sera were titered for interferon by measuring the inhibition of the cytopathogenic effect on mouse fibroblasts (L929) of vesicular stomatitis virus (Indiana strain). Interferon was expressed as the last dilution of mouse serum which inhibited the cytopathogenic effect in 50 percent of the cultures as calculated by the REED-MUENCH method (REED & MUECH, 1938).