ENDOTOXIC SUBSTANCE OF CRYPTOCOCCUS NEOFORMANS

by

Takashi Kobayashi, Izumi Nakashima & Nobuo Kato

Abstract

In this study an endotoxic substance was extracted from the cells of Cryptococcus neoformans and the physicochemical and biological properties of this substance (Cr-ET) were investigated. In comparison with endotoxin of gram-negative bacteria, the lethality of Cr-ET for mice and chick embryos was low and such biological activities were weak as the pyrogenic effect on rabbits and effects on the leucocyte count and blood sugar level in rabbits. Skin reactions (both primary and Shwartzman reactions) were elicited in rabbits by relatively large dose of Cr-ET. Unlike bacterial endotoxin, hyperreactivity to Cr-ET was not induced in mice by prior infection with BCG.

Introduction

Cryptococcosis of man is one of the most important deep-seated mycosis with high mortality, and about a half of the patients was found to be primarily infected without any basic diseases like leukemia and carcinoma (13). A knowledge of the factors relating to the virulence of Cryptococcus neoformans is essential to a understanding of the disease produced and of host responses to this fungal infection. Although many workers studied with the capsular substance of C. neoformans, little studies have been done on the existence of endotoxin of this fungus (1). In this paper, we describe the results of study of the isolation and physicochemical and biological properties of a cryptococcal endotoxic substance.

Materials and methods

Strains

C. neoformans Duke, N-1 and N-2 atrains were used. The Duke strain was supplied by Dr. H. Kobayashi, Chest Disease Research Institute, Kyoto University. The N-1 and N-2 strains were the stock strains of this laboratory. BCG was supplied by the Japanese Tuberculosis Preventive Society, Tokyo.

1 Department of Bacteriology, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan.
Accepted for publication: 7. VII. 1973
Animals

Male SMA mice, 4 weeks old, and female albino rabbits weighing about 2.5 kg were used.

Chick embryos

Eleven-day-old embryonated eggs were used.

Preparation of an endotoxic substance of C. neoformans (Cr-ET)

*C. neoformans* was cultivated at 37 °C for 4 days in Sabouraud’s broth containing 3% of glucose, 200 μg/ml of streptomycin and 200 units/ml of penicillin. The organisms were killed by the addition of formalin to a final concentration of 0.4%, followed by further incubation at 37 °C for 48 hours. The organisms were collected by centrifugation at 7500 rpm for 30 minutes and washed 3 times with M/100 phosphate-buffered saline (PBS). The organisms were dried by washing 3 times with acetone. Dried organisms were suspended in PBS to a concentration of 0.1%. The suspension was sonicated (10 kc, Reytheon sonic oscillator) for 20 minutes. The sonicated preparation was used as the starting material (sonic lysate). Cr-ET was extracted according to the phenol-water procedure (28). The water phase obtained after centrifugation at 3000 rpm for 30 minutes were poured into a dialysis bag and dialyzed against tapped water for 48 hours. To the fluid an equal volume of ethanol was added and the precipitate was removed by centrifugation at 3000 rpm for 30 minutes. The supernatant fluid was concentrated approximately 10-fold in vacuo at 37 °C, and 6 volumes of ethanol and sodium acetate at a final concentration of 5% were added. The precipitate which settled overnight in the refrigerator was collected by centrifugation at 3000 rpm for 30 minutes, the supernatant was discarded, the precipitate was dissolved and dialyzed in water. Precipitation with ethanol and dissolution of precipitate were further repeated twice. The precipitate was dissolved in distilled water and centrifuged at 100,000 G for 2 hours. The sediment was lyophilized and dissolved in PBS prior to use.

Chemical analyses

Proteins were determined by the method of Lowry et al. (23), nucleic acids by UV absorption (34), phosphorus by Bloor’s method (8), lipids (bound) by the method of Westphal et al. (35). The quantitative determination of sugars was performed by Dubois’s method with galactose as the standard (10). For the analysis of sugars by paper chromatography, Cr-ET (0.5 mg) was hydrolyzed in 1 ml of 1 N HCl at 100 °C for 1 hour in a sealed tube, the hydrolysate which was freed from HCl was dissolved in water (0.1 ml) and applied for ascending paper chromatography on Whatman No. 1 paper at 25 °C using the solvent with the composition of