The Anticryptococcal Factor of Blood Serum

ARTICLE *in* JOURNAL OF INVESTIGATIVE DERMATOLOGY · FEBRUARY 1966

Impact Factor: 7.22 · DOI: 10.1038/jid.1966.47

3 AUTHORS, INCLUDING:

George Szilagyi
Albert Einstein College of Medi…

13 PUBLICATIONS **114** CITATIONS

SEE PROFILE

THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 46, No. 3

Printed in U.S.A.

Preliminary and Short Report

THE ANTICRYPTOCOCCAL FACTOR OF BLOOD SERUM

A PRELIMINARY REPORT*

GEORGE SZILAGYI, M.D.,** FREDERICK REISS, M.D.** AND JOHN CRISPIN SMITH, PH.D.***

Reports (1, 2, 3) in the literature indicate that human blood sera inhibit the growth of *C. neoformans*. There are also indications that persons with malignant diseases are prone to infection with this organism (4, 5, 6). In view of the aforementioned we will attempt to determine variations of anticryptococcal factors in healthy persons as well as in persons with malignancies. The present report concerns the anticryptococcal factor of clinically healthy persons, and an attempt of its determination and characterization.

METHODS AND MATERIALS

Clotted human sera randomly obtained from clinically healthy persons were sterilized by passing through a Swinney hypodermic adapter. The sera were prefiltered through a microfiber glass. This was followed by filtration through a Millipore filter, with pores of 0.45 μ diameter. Sera were stored in the frozen state. The test organism was isolated from a human case of cryptococcosis and grown on Sabouraud dextrose agar.† The culture was harvested, washed twice and suspended in Littman's cryptococcus capsule medium. The suspension was adjusted to 0.5 optical density, at 650 millimicron in a Coleman Junior Spectrophotometer. Littman's cryptococcus capsule broth was used as a basal medium for the demonstration of growth inhibition of *C. neoformans* by human serum.

a. The effect of serum on growth of C. neoformans

Serum was added in amounts varying from 0.1 to 1.0 ml to Littman's capsule broth so that the final volume was 3.5 ml. These tubes were inoculated with 0.1 ml of spectrophotometrically standardized *C. neoformans*† suspension. This was incubated at 37°C and agitated twice daily. Growth rate was determined every second day for the first three weeks by reading the optical density in a Coleman Junior Spectrophotometer. Readings were made after the instrument was adjusted to 100% transmission with blank (basal medium mixed with serum) in optical system. Control growth rates were determined as above except that the serum was omitted from the media. To keep the volumes identical, 3, 5, ml of Littman's capsule broth was used. This procedure was applied in studies outlined under b, c, e.

b. The effect of temperature on growth inhibitory factor

In these experiments conditions were kept as under (a.) except that the blood serum was preheated at 37°C, 56°C, and 62°C for 30 minutes before adding to the culture medium.

c. The role of iron binding factor in growth inhibition of C. neoformans *by normal human serum*

In order to determine whether the inhibitory property of serum is due to its iron binding factor, the following tests were carried out:

1. Serum, which in a previous test was found to be strongly inhibitory, was used in this test. Culture medium was prepared as under (a.) except that ferrous ammonium sulfate was added in such amounts, that each tube contained the equivalent of 5 mcg of iron.

2. The same as in (1.) except that the serum used was weakly inhibitory.

d. The role of specific antibodies in growth inhibition of C. neoformans *by normal human Serum*

Cryptococcus agglutination test was carried out on 7 sera (5 showed inhibition of *C. neoformans* and 2 did not) according to the method recommended by Dr. Morris Gordon of N. Y. State Dept. of Health.

e. Inhibitory effect of serum protein fractions

Serum protein fractions obtained by column electrophoresis on cellulose were used in this test. The protein fractions so obtained were dialyzed against water and then lyophilized. When used in the test they were redissolved in 0.85% saline solution. The protein fractions tested were: prealbumin, albumin, albumin + alpha 1 globulin, alpha 2 globulin, beta globulin, gamma 1 and gamma 2 globulins. Tests were conducted as in (a.) except that protein fractions were substituted for serum.

RESULTS

The results obtained with 14 sera randomly collected and tested for inhibition are given in Table

* From the Mycology Research Laboratory** (Division of Medicine), and the Anesthesiology Research Laboratory,*** Montefiore Hospital and Medical Center, Bronx, New York 10467.

Received for publication October 29, 1965.

† The cryptococcus culture was offered through the courtesy of Dr. Marguerite Silva, Chief of Medical Mycology Laboratory, College of Physicians and Surgeons, Columbia University, New York, for which we express our gratitude.

I. A 50% inhibition of growth was considered significant. The maximum inhibition of growth occurred between the sixth and eighth day of incubation. After that period there was a rapid increase in growth, approaching that of control.

To produce 50% inhibition at least 13% serum had to be incorporated in the medium. The inhibition is shown in Fig. 1. Complete inhibition was not obtained by increasing the amount of serum added to the culture medium. The effect of heat treatment on inhibition is shown in Fig. 2. The growth at room temperature and at 37°C was the same. Preheating the serum to 56°C for 30 minutes slightly reduced its inhibitory activity. Heating the serum to 62°C for 30 minutes destroyed its inhibitory activity.

Fig. 3. gives the results of addition of iron to serum on its inhibitory activity. Adding 5 mcg of iron per assay to a very active serum, reversed its inhibitory property. Adding the same amount of iron to a low inhibitory serum, did not reverse the inhibition. From these data one may assume that

TABLE I

Illustrating the rate of inhibition by randomly collected sera from healthy subjects

Serum #	% Inhibition
63	21
7	25
5	29
17	38
15	42
6	42
62	50
65	54
72	54
18	57
19	60
13	63
14	67
82	71

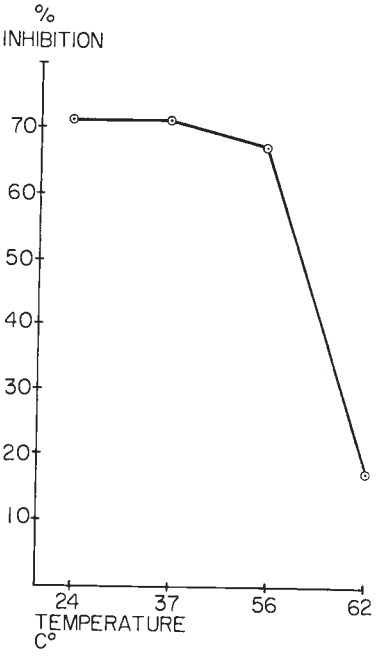

FIG. 2. The effect of temperature on growth inhibitory activity of human serum.

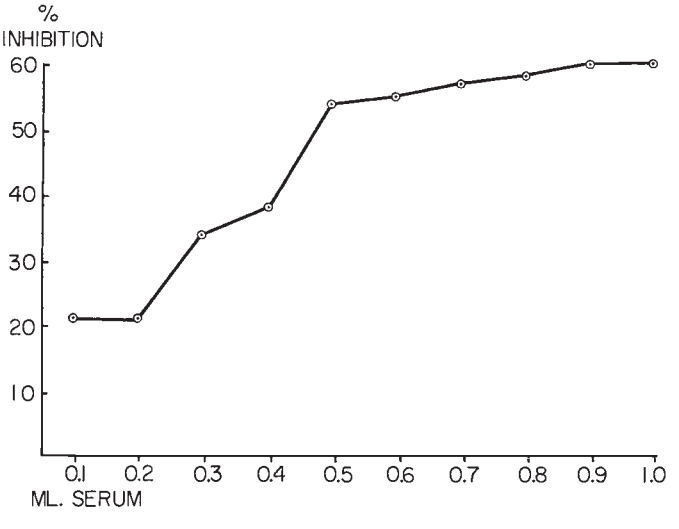

FIG. 1. The effect of serum concentration on growth inhibition of *C. neoformans*

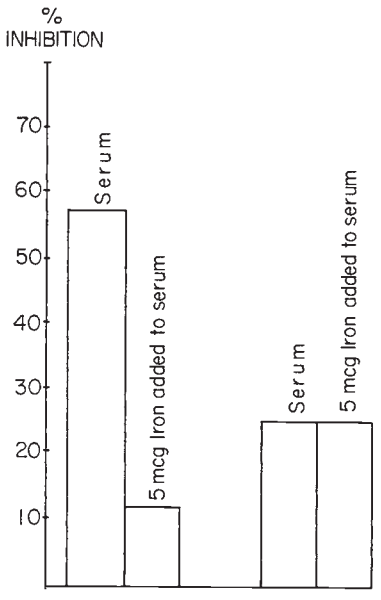

FIG. 3. Reversal of inhibitory activity by iron

TABLE II

The rate of anticryptococcus activity of various serum protein fractions

Protein fraction	% Inhibition
Whole serum	60
Prealbumin	10
Albumin	6
Albumin + alpha 1 globulin	6
Alpha 2 globulin	73
Beta globulin	0
Gamma 1 globulin	7
Gamma 2 globulin	80

in addition to the iron binding factor of serum, (7) other factors may be involved in the inhibitory activity of serum.

Growth inhibition of *C. neoformans* by serum protein fractions is presented in Table II. It was observed that the fractions with significant activity are found in the regions of alpha 2 and gamma 2 globulin.

No specific antibodies were demonstrated by cryptococcus agglutination test performed on both inhibitory and non-inhibitory sera.

Further investigations are being carried out to demonstrate the presence of the anticryptococcal factor in blood of different mammalian species and patients with malignancies and, furthermore, to determine exactly the factor or factors responsible for growth inhibition of *C. neoformans*.

REFERENCES

1. Allen, W. P.: An investigation of the anticryptococcal properties of normal serums. Doctoral Dissertation, University of Michigan, Ann Arbor, Michigan, 1955.
2. Baum, G. L. and Artis, D.: Growth inhibition of Cryptococcus neoformans by cell free human serum. Amer. J. Med. Sci., *241:* 613, 1961.
3. Baum, G. L. and Artis, D.: Characterization of the growth inhibition factor for Cryptococcus neoformans (GIFe) in human serum. Amer. J. Med. Sci., *246:* 53, 1963.
4. Gendel, B. R., Ende, M. and Norman, S. L.: Cryptococcosis; a review with special reference to apparent association with Hodgkin's disease. Amer. J. Med., *9:* 343, 1950.
5. Collins, V. P., Gelhorn, A. and Trimble, J. R.: The coincidence of cryptococcosis and disease of the reticuloendothelial and lymphatic system. Cancer, *4:* 883, 1951.
6. Zimmerman, L. E. and Rappaport, H.: Occurrence of cryptococcosis in patients with malignant disease of the reticuloendothelial system. Amer. J. Clin. Path., *24:* 1050, 1954.
7. Caroline, L., Taschdjian, C. L., Kozinn, P. J. and Schade, A. L.: Reversal of serum fungistasis by addition of iron. J. Invest. Derm., *42:* 415, 1964.