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**DEPARTMENT OF THE ARMY**

Fort Detrick

Frederick, Maryland
IMMUNOLOGICAL STUDIES ON CRYPTOCOCCOSIS

REPORT II. STUDIES ON THE SPECIFICITY OF THE POLYSACCHARIDE ANTIGEN OF CRYPTOCOCUS NEOFORMANS IN EXPERIMENTAL CRYPTOCOCCOSIS

In the previous report, the author described the extracting and purifying method of polysaccharide fraction from Cryptococcus neoformans and its chemical nature. In that report the author also suggested that the immunological diagnosis of cryptococcosis can be applied clinically by performing the precipitin reaction and skin test. Also, the author investigated the degree of manifestation of cross immunological reaction in which the polysaccharide fraction of Cr. neoformans was used as an antigen to the animal infected with Candida or Saccharomyces, both of which presumably possessed a somewhat common antigen as Cr. neoformans. The investigation was further carried out as to how the anamnestic reaction would re-appear when a rabbit previously immunized by Cr. neoformans was re-immunized after its antibody in blood had disappeared. The latter investigation was also aimed at clarifying the immunization mechanism of Cr. neoformans.

METHODS AND MATERIAL

I. Anamnestic immunological reaction:

Animals used: rabbits no. 8, 9, and 10 used in the previous experiment.

Strain used: Cr. neoformans no. 2617 and no. 2575 strains.

Method: The rabbits whose antibody titers (precipitin titers) and skin reactions became negative following the previous experiment were re-immunized. Cr. neoformans organisms were collected after being cultivated on 3 percent dextrose, Sabrond's agar-agar slant media at 37°C for 48 hours and were heated at 60°C for thirty minutes after being diluted with...
physiological saline to contain 1 mg./ml. Each ml. of the obtained solution was injected into the rabbit's ear vein repeatedly. The degree of an elevation of the antibody (precipitin titer) titer in the blood sampled from the rabbits and the degree of manifestation of skin reactions in relationship to the blood antibody-level were frequently examined during the process of experiment.

RESULTS:

As shown in Illustration 1, the degree and promptness of the manifestation of the precipitin reaction varied depending on the individual differences in the rabbit. But, in each rabbit, some degree of the anamnestic reaction was noticed in their sera, although no anamnestic skin reaction was recognized in any of the rabbits.

II. Cross immunological reaction among the similar organisms:

Animals used: Rabbits weighing approximately two kgs.

Strain used: Cr. neoformans, no. 2617 strain, Candida albicans, no. 2 strain, Candida krusei, no. C-4-1 strain and Saccharomyces cerevisiae, no. 2235 strain.

Each organism was collected after being cultivated on three percent dextrose Sabrland's agar-agar slant media at 37°C for 48 hours, and was heated at 60°C for thirty minutes after being diluted with physiological saline to contain 1 mg./ml. One ml. of the obtained solution was injected into each rabbit's ear vein repeatedly.

During the process of the experiment, the antibody-titers of the vaccinated rabbits were frequently measured. Among them, the antibody titer of C. albicans immunized rabbit was measured by precipitin reaction in which the polysaccharide fraction extracted from C. albicans no. 2 strain was utilized as its antigen (2). On the other hand, the antibody of the C. krusei and of the S. cerevisiae were titrated by the agglutination reaction. Furthermore, each immunized rabbit's serum was checked for a precipitin reaction by using the polysaccharide fraction extracted from Cr. neoformans no. 2617 strain as an antigen.

RESULTS:

Variations of the precipitin titers of Cr. neoformans and of the similar organism-immunized rabbits are shown in Illustration 2.

For example, the precipitin reaction in which the polysaccharide fraction extracted from Cr. neoformans was used as an antigen was
particularly increased in the serum of Cr. neoformans immunized rabbit. On the other hand, in the immunized sera of C. albicans, C. krusei and of S. cerevisiae, the precipitin reaction with Cr. neoformans extracted polysaccharide fraction was positive only in the later period of immunization with as high a concentration of the polysaccharide fraction as 1000 mcg./ml.

With regard to the skin reaction, the rabbits immunized by C. albicans, C. krusei and by S. cerevisiae manifested a redness of about (1-5mm) x (1-5mm) in size at 10 mcg./ml. of its concentration.

Variations of the precipitin reactions of C. albicans immunized rabbits in which the C. albicans extracted polysaccharide fraction and the extracted Cr. neoformans were used, are shown in Illustration 3. Also shown in Illustration 3, the precipitin reaction of the serum of the C. albicans immunized rabbit against which the polysaccharide fraction extracted from C. albicans was matched, was particularly increased. However, its precipitin reaction against which Cr. neoformans extracted polysaccharide fraction was matched, was slightly increased only in the later period of the immunization.

DISCUSSION:

Some degree of anamnestic reaction of the cryptocooccal infection was provoked by the precipitin reaction. However, it was not marked in its degree and elevation of its antibody titer was extremely slow at the onset when initial immunization was performed. Also, when it was re-immunized following the disappearance of its antibody from the blood, it was as slow and unmarked as the initial one. Generally, its reaction was much slower than that of the bacterial immunization. Moreover, the rate of disappearance of antibody from the blood when re-immunized was much faster than that of the initial immunization. Positive skin reactions were not revealed in any of the three re-immunized rabbits. These findings revealed that re-immunization was quite contrary to that of initial immunization in which the degree of variation in its skin reaction was parallel to those of the precipitin reaction.

The reason for the dulness of the skin reaction found in the serum may be due to the fact that dead organism was used at its re-immunization. Further investigation is required to prove whether or not positive skin reaction can be obtained with the use of the live vaccine. Although it cannot be said without reserve, that such findings as seen in Cr. neoformans immunization can be applied to other mycoses, the attitude of the mycoses in general towards immunization should be different from that of bacteria. It can be concluded that some common antigen does exist among C. albicans, C. krusei, S. cerevisiae and Cr. neoformans.
since a cross reaction was found among their sera in which the precipitin reaction was used as its antigen. Evans and Sorensen (3) reported on animal experiments in which Cr. neoformans and some of the other mycoses proved to possess a common antigen. They used the agglutination and precipitin reaction to prove it.

Salvin (4, 5) also reported the cross reaction between Cr. neoformans and other mycoses in which the complement fixation was utilized.

Although it is well recognized that a common antigen exists between Cr. neoformans and other similar mycoses, the highly specific reaction against polysaccharide fraction extracted from Cr. neoformans was noticed only in the Cr. neoformans immunized serum which showed a marked qualitative difference between their reactions.

These findings suggest that the polysaccharide fraction extracted from Cr. neoformans can be utilized for the purpose of differential diagnosis.

CONCLUSION:

1. In order to investigate the specificity of the polysaccharide fraction extracted from Cr. neoformans no. 2617, cross immunological reactions among the rabbits sera immunized by Candida albicans, Candida krusei, Saccharomyces cerevisiae and Cr. neoformans were investigated. The precipitin reaction was utilized for this purpose. As a result of this experiment, a positive cross immunological reaction among them was recognized to a slight degree.

2. It was proved that the anamnestic immunological reaction could be recalled by re-immunizing the rabbit when its antibody titer in blood was decreased and skin reaction became negative sometime after immunization with Cr. neoformans.
Illustration 1. Variation of the precipitin reaction and skin reaction of the Cr. neoformans no. 2575 infected rabbit to which the polysaccharide fraction was utilized as its antigen and of its anamnestic reaction.

Key: 1 — (mcg/ml); 2 — Dead vacc.; 3 — Live vacc.; 4 — mcg; 5 — Precipitin reaction; 6 — Skin reaction; 7 — No. of months.

Illustration 2. Variation of the precipitin reaction of the Cr. neoformans infected rabbit and of similar mycoses infected rabbits to which the Cr. neoformans extracted polysaccharide was utilized as its antigen.

Key: 1 — Amt. of Cr. neof. extract. polysacch. fraction (mcg/ml); 2 — Cr. neof. immun.; 3 — C. albicans immun.; 4 — S. Cerevisiae immun.; 5 — C. krussi immun.; 6 — No. of months.
Illustration 3. Variation of the precipitin reaction of the C. albicans immunized rabbit to which the C. albicans extracted polysaccharide fraction was used as its antigen and of the Cr. neoformans immunized rabbit to which extracted Cr. neoformans was used as its antigen.

Key: 1. Dose of polysaccharide fraction (mcg/ml); 2. C. albicans P.S.F.; 3. Cr. neoformans P.S.F.; 4. No. of months.

REFERENCES