ELECTROPHORETIC ANALYSIS OF *TRYPANOSOMA GAMBIENSE* ANTIGENS CONCERNED WITH AGGLUTINATION AND PROTECTION

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INTRODUCTION

There are generally considered two groups of antigens in trypanosomes. One group consists of common antigens and the other group, those specific to the strain. Specific antigens are those concerned with agglutination or protection against infection (Weitz, 1960; Seed, 1963).

Antigens of trypanosomes have been fractionated by a variety of methods, such as differential centrifugation (Brown and Williamson, 1964), ammonium sulfate fractionation (Brown and Williamson, 1964; Seed, 1972), DEAE-Sephadex (Seed, 1972), and gel filtration (Mahmoud and Kreier, 1972).

The present paper aims to report the fractionation of the specific antigens by means of block electrophoresis, and their properties as regards agglutination and protective abilities.

MATERIALS AND METHODS

Parasites: The Wellcome strain, antigenically 0 type *T. gambiense*, maintained in mice (dd strain) 18-20gm by weight and serially transferred at three days interval, was used throughout the experiment.

Collection and separation of organisms: For this study, Wistar male rats were experimantally infected intraperitoneally (IP) with 5×10^7 parasites from a mouse infected three days earlier. The blood was collected three days after infection by heart puncture. Parasites free of host blood cell components were obtained by the method of Lanham and Godfrey (1970). The parasites were washed 3 times with 1% glucose phosphate buffer (GPB), $\mu = 0.271$, pH 7.5, centrifuged at 800 × G for 8 min, and then homogenized in a sonicator (20KC) for 5 min. After centrifugation at 15,000 × G for 60 min, the supernate was stored at -25°C until used as antigen.

Block electrophoresis: Samples were dialyzed overnight against veronal buffer, $\mu = 0.05$, pH 8.6 at 4°C. Denaturated protein, if present, was removed by centrifugation. The supernate was applied to a 2.5 × 7 × 0.1 cm agar gel plate. A direct current of 5 mA/plate for 5 hours at 4°C was applied. Two millimeter-wide fractions were cut and extracted by freeze and thawing method. Nitrogen measurements of fractions were done by the microkjeldahl method (Kabat and Mayer, 1961).

Immunization: Each fraction from block electrophoresis was injected IP to four mice, respectively. After 10 days, two mice from each group were bled from the heart. The sera were inactivated at 56°C for 30 min and stored at −25°C until used. The remaining two mice from each group were used to test the protective ability of the different fraction.

Agglutination test: Two-fold dilutions of the serum (0.5ml) were mixed with equal volume of GPB containing 1×10^8 trypanosomes. After 10 min, the agglutination titer was determined as the last dilution of anti-

serum in which agglutination occurred. Observations were done under the microscope.

Protective ability against experimental inoculation of parasites: After 10 days, mice immunized against each of the fractions were inoculated with 1×10^4 trypanosomes. They were then examined for the presence or absence of the parasites for a period of 8 days after inoculation.

RESULTS

Fractionations by block electrophoresis have yielded two groups each with more nitrogen content than the others (Fig. 1).

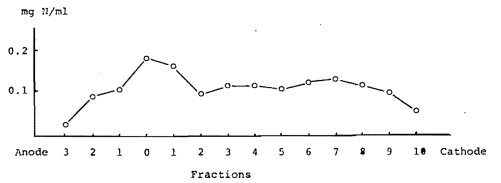

Fig. 1—Nitrogen measurement of the fractions by means of block electroporesis.

Agglutination tests made of the antiserum against each of the fractions showed that the same two groups are distinctly positive (Table 1). Similarly, tests on the protective ability from the two groups gave positive results. Complete protection against experimental infection was shown by the two distinct fractions showing the highest amount of nitrogen and the highest agglutination titer. The other fractions in each of the two groups, although unable to completely protect the host against infection, showed a delay in the onset of parasitemia and the occurrence of death.

DISCUSSION

The antigenic components of *T. gambiense* concerned with agglutination separated into two groups electrophoretically. The same two groups showed the capacity to protect the host from experimental infection. The antiserum against the fraction in each group

Table 1

Antigenicity of the fraction to the agglutination and the protection.

Fractions	Agglutination titer of the antiserum against the fraction	Days of parasitemia of the mouse immunized with the fraction	Days of death of the mouse immunized with the fraction
+3	0	2	5
+2	0	2	5
+1	0	2	5
0	1/16	-*	-**
−1	1/4	3	6
−2	0	2	5
−3	0	2	5
−4	0	2	5
−5	0	2	5
−6	1/2	3	5
−7	1/8	-	-
−8	1/4	3	6
−9	1/2	3	5
−10	0	2	5

* = No parasitemia, ** = Protection against infection,
+ = Positive side, − = Negative side, 0 = Original region for sampling.

showing the highest agglutination titer gave also complete protection. From the above, the agglutination antigen is the same as the protective antigen. In addition, the agglutination antigen presumably does not exist as a simple substance but rather as a complex protein separable by electrophoresis. Undoubtedly, other more refined methods of fractionation are necessary to characterize the agglutination antigen completely.

SUMMARY

This report deals with the fractionation of the antigenic components of *Trypanosoma gambiense* by means of block electrophoresis, and the serological analysis of electrophoretic fractions as regards agglutinating and protective abilities. Antigens concerned with agglutination separated into two groups of fractions, which also showed protection against infections. Both agglutination and protective antigens showed the same electrophoretic patterns and were extracted from the same fraction. It might be considered that the agglutination antigen(s) and protective one(s) are the same.

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