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Determination of IgG Antibodies Produced by the Kinetoplast Fraction Antigens of Trypanosoma Rhodesiense, T.Vivax and T. Brucei by Enzyme linked Immunosorbent Assay

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SUMMARY

Antibodies to trypanosome homegenate and subcellular particles of different trypanosome species, with emphasis on the kinetoplast fraction, were measured by the ELISA method. The antibodies formed to these homogenates and subcellular particles could be titrated; the kinetoplast fraction, particularly, not only gave titration against a heterologous strain, but also showed activity against this strain after two to three antigenic variations.

INTRODUCTION

African trypanosomiasis has immunological phenomena which manifest in parasite antigenic variation and host antibody production. The parasite may be considered to have two sets of antibody producing fractions (Gray, 1967; de Raadt, 1974): one, the surface or variant antigens which elicit IgM antibodies, and two, internal, particulate or common antigens which elicit IgG (de Raadt, 1974). These common antigens also give some immunoprotection to heterologous strains (Powell, 1976a). There is some evidence that the immunoprotective effects of the common antigens reside in the kinetoplast fraction (Powell, 1976b).

We felt that it would be of interest to have some knowledge of the relative amounts of antibody that might produce these effects. Enzyme-linked immunosorbent assay (ELISA) is a micro quantitative antibody or antigen assay comparable to radio immunosorbent test (RIST) (Engvall and Parlmann, 1972). It has been used in the diagnosis of Salmonella infection (Carlsson et al., 1972, 1975) and Chagas' disease (Voller et al., 1975). This paper describes the use of ELISA to estimate the titer of trypanosomal homogenate and kinetoplast antibody activity.

MATERIALS AND METHODS

Trypanosoma vivax (Desowitz) and T.vivax (Liverpool) were donated by Dr. W.E. Ormerod and Dr. S.C. Langham, London; T.rhodesiense (CRS 41) and T.brucei (CRS 2) were donated by Dr. M.A.Q. Awan, Mazabuka.

The methods of antigen isolation and antibody formation were performed essentially as previously described (Powell, 1976a). Homogenate and kinetoplast fractions were used as antigens unless otherwise specified.

Rats were injected i.m. in the foot pads and i.p. on the backs on days 0, 5, 9, 14, and bled three days later. The serum was collected and used as antibody.

ELISA was performed essentially as described by Engvall and Perlmann (1972).

RESULTS

Determination of the IgG antibodies produced by the subcellular fractions of T.rhodesiense. Plastic tubes were incubated with isolated fractions of T. rhodesiense, and the ELISA method was used. Antibody to T.rhodesiense homogenate was used as antibody with rabbit anti-rat antibody conjugated to alkaline phosphatase. The results are summarized in Table 1. No antibody gave no results, as did no antigen. The antigen with the highest activity was that of the kinetoplast.

Titration of the IgG antibodies. Tables 2 and 3 describe titration of the IgG antibodies of anti-homogenate T.rhodesiense against homogenate T.

TABLE I

Average IgG activity (3 assays) in T.rhodesiense fractions against anti-homogenate T.rhodesiense.

Fraction	O.D. (400mn) /100 min /mg protein	S.D.	%
Homogenate	2.088	±0.080	–
Cell wall	2.218	±0.043	18.3
Nuclei	2.665	±0.070	21.9
Kinetoplast	3.646	±0.047	30.0
Mitochondria	0.920	±0.035	7.6
Microsomes	1.342	±0.042	11.0
Cell supernatant	1.357	±0.021	11.2

TABLE II

Titation of the average IgG activity (3 assays) of anti-homogenate T.rhodesiense against homogenate T.rhodesiense.

Antibody dilution ratio	O.D./100 min/mg protein	S.D.
1:1	1.400	± 0.225
1:10	1.396	± 0.210
1:100	0.793	± 0.035
1:1000	0.680	± 0.048

rhodesiense and anti-kinetoplast T.rhodesiense against isolated T.vivax (Desowitz).

Determination of the IgG antibodies of homogenate and kinetoplasts of T.vivax (L) and T.rhodesiense. Table 4 presents a summary of the IgG antibody activity as determined by ELISA against homologous and heterologous strains. It can be noted that anti-homogenate T.vivax (L) has 91.8% of the activity of anti-homogenate T.rhodesiense against homogenate T.rhodesiense, and that of the anti-kinetoplasts of T. vivax (L) and T.rhodesiense have between 80% and 95% of the anti-homogenate's activity.

Determination of the IgG antibodies to T. brucei and T.vivax (D) by anti-homogenates and anti-kinetoplasts of T.vivax (L) and T.rhodesiense. Table 5 summarizes the IgG activity as determined by ELISA during antigenic variation. It can be noted that in T. brucei, while the anti-homogenates and anti-kinetoplasts from T.vivax and T.rhodesiense gave comparable results to T.brucei, by the second antigenic variation the anti-homogenate activity had dropped considerably, while the anti-kinetoplast activity from both antibody fractions remained fairly constant. With T. vivax (D), after three antigenic variations, the anti-kinetoplast. T.rhodesiense still gave strong IgG activity to T.vivax.

DISCUSSION

These studies demonstrate that ELISA can be used to quantitate IgG antibody to trypanosomes.

The studies also demonstrate that the subcellular fractions have greater IgG antibody activity, which

TABLE III

Titration of the average IgG activity (3 assays) of T.vivax (D) homogenate against anti-kinetoplast T.rhodesiense, with comparative values for the 1:100 titration of T.vivax (D) and T.rhodesiense.

Antigen		Antibody to		Dilution ratio	O.D./ 100 min/ mg protein	S.D.
vivax	homogenate	rhodes	kinetoplast	1:1	2.045	±0.240
vivax	homogenate	rhodes	kinetoplast	1:10	1.724	± 0.140
vivax	homogenate	rhodes	kinetoplast	1:100	0.627	± 0.032
vivax	homogenate	rhodes	kinetoplast	1:1000	0.056	± 0.008
vivax	isolate	rhodes.	kinetoplast	1:100	0.321	± 0.056
vivax	isolate	vivax	kinetoplast	1:100	0.477	± 0.024

TABLE IV

Average IgG activity (6 assays) of T.vivax (D) and homogenates of T.vivax (L) and T.rhodesiense against anti-homogenate and anti-kinetoplast T.vivax (L) and T.rhodesiense, with 1:1 titration ratio.

Antigen	Antibody to	O.D./100 min /mg protein	S.D.
Vivax (L) homog	vivax (L) homogenate	2.167	± 0.240
rhodes homog	rhodes homogenate	3.370	± 0.232
rhodes homog	vivax (L) homogenate	3.091	± 0.650
vivax (L) homog	vivax (L) kinetoplast	2.070	± 0.210
rhodes homog	rhodes kinetoplast	3.033	± 0.130
rhodes homog	vivax (L) kinetoplast	2.734	± 0.100
vivax (D) isolate	rhodes kinetoplast	2.095	± 0.095

TABLE V

Antigen Variation	T.brucei 1	2	1	T.vivax (D) 2	3
anti-vivax (L) homogenate	0.4410 (±0.070	0.1920 (±0.032)			
anti-vivax (L) kinetoplast	0.4635 (±0.088)	0.5865 (±0.032) t = 2.795			
anti-rhodes homogenate	0.7680 (±0.043)	0.1260 (±0.021)			
anti-rhodes kinetoplast	0.4698 (±0.024)	0.4860 (±0.042) t = 3.210	0.4950 (±0.028)	0.2962 (±0.035)	0.7832 (±0.450)
no of rats tested	6	6	6	5	5
no of tryps /25 fields	176	73	68	244	109

is consistent with other findings (de Raadt, 1974).

It is significant that the antibody to T.rhodesiense kinetoplast, which has a somewhat higher activity among the subcellular fractions, can be used to titrate T.vivax (D) homogenate with a similar titration as anti-homogenate T.rhodesiense to homo genate T.rhodesiense (Tables 2 and 3). It is also of interest that anti-kinetoplast T.rhodesiense is effective against isolated T.vivax (D) (Table 4). Although anti-homogenates of T.vivax (L) and of T.rhodesiense gave antibody activity against T.brucei (Table 5), of even greater importance is the evidence that the antibody to kinetoplast of both strains but was also effective after antigenic variation in heterologous strains.

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REFERENCES

Carlsson, H.E., Lindberg, A.A. and Hammarstrom, S. (1972). Filtration of antibodies to Salmonella O. antigens by enzyme-linked immunosorbent assay. Infection and immunity 6(5): 703–708.

Carlsson, H.E., Lindberg, A.A., Hammarstrom, S. and Ljungren, A. (1975). Quantitation of Salmonella O. antibodies in human sera by enzyme-linked immunosorbent assay (ELISA). International archives of allergy and applied immunology 48: 485–494.

Engvall, E. and Perlmann, P. (1972). Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen coated tubes. Journal of immunology 109 (1): 129-135.

Gray, A.R. (1967). Some principles of the immunology of trypanosomiasis. Bulletin of the World Health Organisation 32: 177–193.

Powell, C.N. (1976a). Immunoprotective effects of bound particulate subcellular fractions of Trypanosoma brucei and T. rhodesiense. Medical Journal of Zambia 10 (2): 27–32.

Powell, C.N. (1976b). Identification of an immunoprotective subcellular fraction of Trypanosoma brucei and T. rhodesiense. Medical Journal of Zambia 10(2): 32–34.

Raadt, P. de (1974). Immunity and antigenic variation: clinical observations suggestive of immune phenomenon in African trypanosomiasis. In: Trypanosomiasis and Leishmanisasis with special reference to Chagas' disease, Ciba Foundation symposium, new series, 20, p.199-224. Amsterdam, Elsevier.

Voller, A., Draper, C., Bidwell, D.E. and Bartlett, A. (1975). Microplate enzyme-linked immunosorbent assay for Chagas' disease. Lancet I (Feb. 22): 426–430.