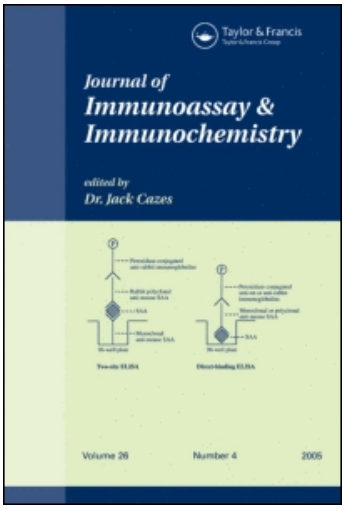

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An Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Trypanosomal Antigens in Goat Serum Using a Monoclonal Antibody

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AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF TRYPANOSOMAL ANTIGENS IN GOAT SERUM USING A MONOCLONAL ANTIBODY

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(KEY WORDS: Monoclonal antibody; Trypanosoma brucei evansi; ELISA; trypanosomal antigens; African Trypanosomiasis).

ABSTRACT

An IgM murine monoclonal antibody (MAb) TEA 1/23.3.4.6 raised against circulating trypanosome antigens was used in a sandwich ELISA to assay trypanosomal antigens in a trypanosome lysate preparation and in sera from goats infected with Trypanosoma brucei evansi. As little as 1.25ug/ml of trypanosomal antigen could be detected by this assay. Following infection, trypanosomal antigens were first detected in goat serum 24 hours after the intravenous (i/v) or 6 days after the intramuscular (i/m) inoculation of trypanosome parasites. Antigen levels remained detectable during the course of infection. After treatment with diminazene aceturate, antigens dropped to undetectable levels between day 12 to 41, suggesting that this assay offers a promising approach to the diagnosis of African Trypanosomiasis.

INTRODUCTION

The occurrence of trypanosomal antigens in the sera of trypanosome-infected animals has been known for a number of

decades (1). Due to the lack of sensitive and safe serological techniques, the exploitation of these antigens for diagnostic purposes was only realised after the advent of enzyme immunoassay techniques (2,3). Since then, there has been a major improvement in the development and application of enzyme immunoassay techniques for the diagnosis of parasitic infections.

Recently, a number of workers have reported success in the application of enzyme immunoassay techniques for the detection of circulating trypanosomal antigens in the sera of infected animals (4,5,6,7,8). In these assays, both polyclonal and monoclonal antibodies have been employed successfully as antigen detecting probes.

In this paper, we report the use of a murine anti-trypanosome monoclonal antibody for the detection of circulating trypanosomal antigens in goats experimentally infected with T.b. evansi KETRI 1342. The monoclonal sandwich ELISA technique developed was sensitive and easy to perform.

MATERIALS AND METHODS

Production of Monoclonal Antibody

Ten adult female Balb/c mice (8-9 weeks old) were each immunized intraperitoneally (i/p) with 0.2 ml of mouse

plasma from heavily parasitaemic Balb/c mice infected with T.b. evansi KETRI 1342. This antigenic material, rich in circulating trypansomal antigens, was first emulsified in complete Freund's adjuvant before immunization. Four weeks later, each mouse was boosted i/p with a 0.2 ml dose of antigen in incomplete Freund's adjuvant, and repeated after another four to six weeks. On each of the last three days before fusion, each mouse received 0.2 ml i/v, of the antigen-rich mouse plasma as recommended by Stahli et al. (9). Spleen cells were harvested from one mouse and fused with mouse myeloma cells (NS-1). Hybridomas producing antibodies with high binding capacity, specificity and affinity for trypanosomal antigens were cloned and characterised as described by Olaho-Mukani et al. (10). A hybridoma secreting murine IgM MAb TEA 1/23.4.6 was selected and grown in pristane (Sigma, USA) - primed Balb/c mice, ascites harvested, and antibody purified on Sepharose 6B as described by Rurangirwa et al. (11). The MAB TEA 1/23.4.6 was found to bind to a somatic non-variant antigen present in trypanosome lysate (10).

Conjugation of MAb TEA 1/23.4.6 to Horse radish Peroxidase (HRPO)

Horse radish peroxidase Type VI (Sigma, USA) was conjugated to TEA 1/23.4.6 according to the method of Henning and Nielsen (12). Briefly, 10 mg of HRPO was

dissolved in 2.5 ml of double distilled deionized water and mixed with 0.5 ml of 0.1M sodium meta-periodate for 20 minutes at room temperature. The aldehyde was dialysed against 1mM sodium acetate buffer PH 4.4 at 4°C for 18 hours. Fifty microlitres of 0.2 M sodium carbonate buffer, pH 9.5 was added to HRPO solution followed immediately by the addition of 2 mg of purified MAb. The mixture was stirred at room temperature for two hours and the reaction stopped by the addition of 0.25 ml of ascorbic acid (4 mg/ml).

Infection of Goats and Preparation of Serum Samples

Two groups of goats (Group 1, five goats, and Group 2, six goats) were infected with $2x10^6$ trypanosomes per goat by syringe and needle i/v inoculation (Group 1) or i/m inoculation (Group 2). Pre-infection and post-infection bleeds were carried out on specified days for serum preparation and quantification of parasitaemia by haemacytometer counting throughout the study period (114 days). Group 1 goats were eventually treated with diminazene aceturate (Berenil, Hoechst, Germany) on day 58 and Group 2 on day 70 post-infection. Serum samples were kept at -20°C until assayed.

ELISA Procedure

Block titration of TEA 1/23.4.6-HRPO conjugate and the TEA 1/23.4.6 were first carried out to determine the optimal

conjugate dilution and the amount of MAb required to coat the microtitre ELISA plates for use in subsequent sandwich ELISA for trypanosome antigen detection. Subsequently, microtitre plates (Dynatech, USA) were coated with 25ug/well of MAb in 200ul/well of carbonate buffer PH 9.6, by incubating plates at 37°C for one hour and overnight at 4°C. The plates were then washed three times (5 minutes/wash) with phosphate buffered saline (PBS) pH 7.4, containing Tween-20. To each well, 200ul of undiluted serum or diluted trypanosome lysate antigen (in PBS) were added and the plates incubated at 37°C for one hour. The plates were washed as before and 200/ul/well of MAb/HRPO conjugate added at 1:1000 dilution. The plates were again incubated for 1 hr at 37°C and washed as before. Lastly, 200ul/well of ortho-phenylene diamine (OPD) (Sigma, USA) was added and the plates incubated at room temperature in the dark for 30 minutes. The enzyme-substrate reaction was stopped by adding 100 ul/well of 1.0 M sulphuric acid. Absorbance at 492 nm was read on a 2-wavelength microplate autoreader (Dynatech MR580, USA). All readings for standard and test samples were made in duplicate.

RESULTS

Detection of Lysate Antigen

Figure 1 shows the concentration of trypanosome lysate antigen detected by MAb TEA 1/23.4.6 in a sandwich ELISA. As shown, as low as 1.25 ug/ml or 250 ng per microwell (200 ul/well) of lysate antigen could be detected. Below this

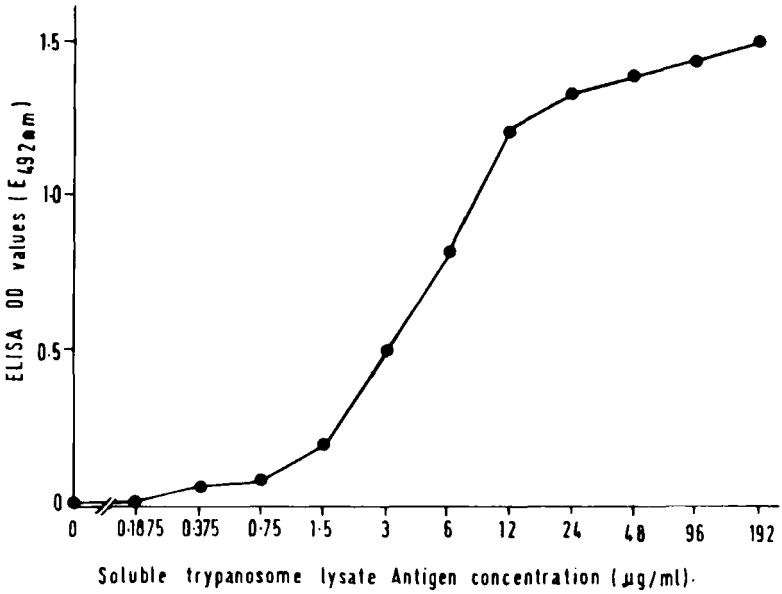

Figure 1: Concentration of trypanosome lysate antigen detected by TEA 1/23.4.6 in a sandwich ELISA.

level, OD readings remained too low to give meaningful interpretation.

Detection of Circulating Trypanosomal Antigens in goat Sera

Figures 2 and 3 show the levels of circulating trypanosome antigen in the two groups of goats during the course of infection. Pre-infection mean (±SE) OD reading for Group 1 goats was 0.029±0.002 while that of Group 2 goats was 0.033±0.004 nm. Twenty four hours after

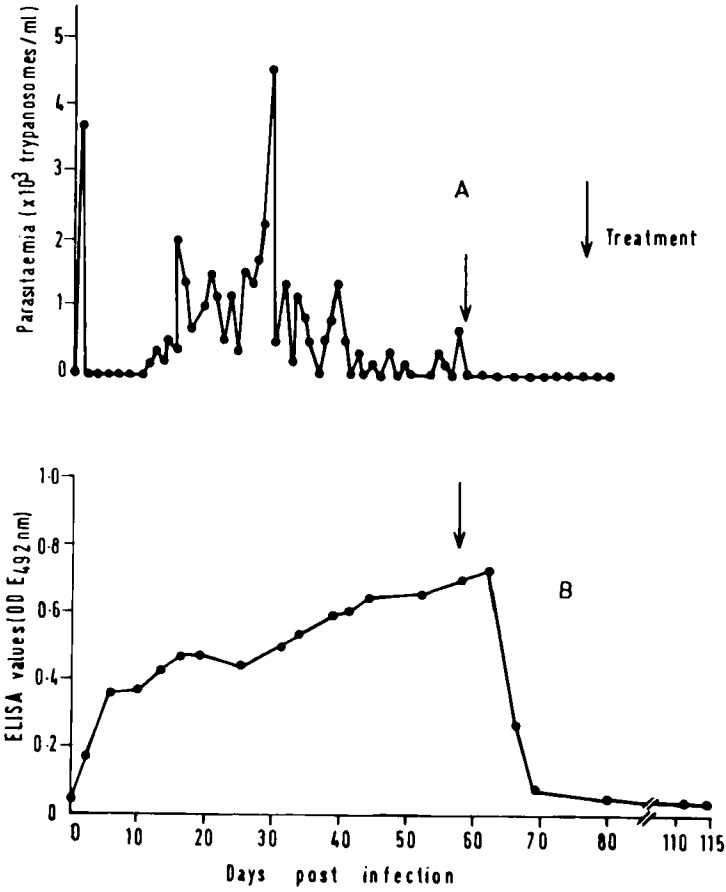

Figure 2: Parasitaemia (x10^3 trypanosomes/ml) and serum Ag-ELISA values (OD, 492nm) in the goats of group 1 during the experimental period.

infection, in Group 1, the levels rose to a mean OD reading of 0.089±.006, coinciding with the appearance of parasitaemia. In Group 2 goats, the levels remained low until day 6, when they rose to a mean level of 0.207±.054,

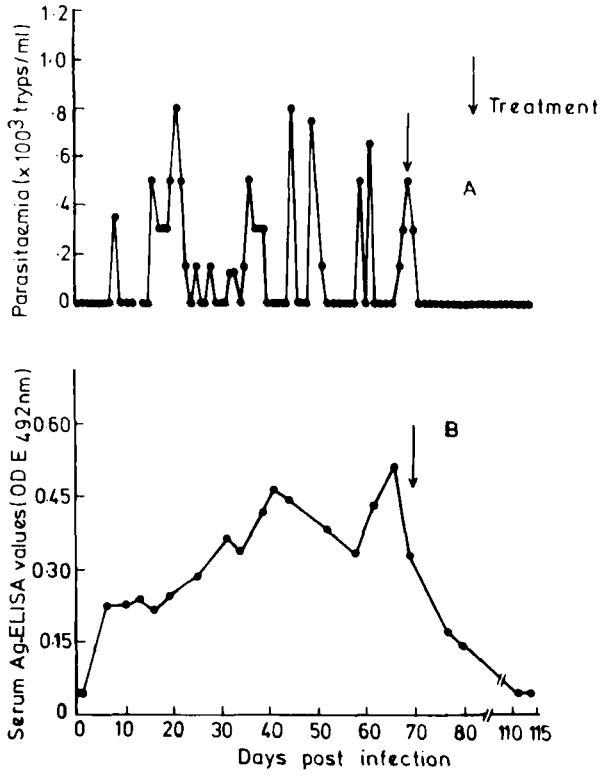

Figure 3: Parasitaemia (x10^3 trypanosomes/ml) and serum Ag-ELISA values (OD-492nm) in the goats of group 2 during the experimental period.

again, conceding with the appearance of parasitaemia. There was a more marked fluctuation in the pattern of parasitaemia in Group 2 goats than in Group 1 goats. This may be due to the different routes of infection used in the two groups. Detectable antigen levels remained high in both groups during the course of infection, with slight fluctuation, reaching the highest level on day 62 in Group 1 (mean OD,

0.719±.035) and in Group 2 on day 66 (mean OD, 0.516±.044). In both groups of goats, antigens could be detected even in the absence of detectable parasitaemia (Figures 2 and 3).

Following treatment with diminazene aceturate, antigen levels dropped to pre-infection levels by day 69 in Group 1 goats (treated on day 58) and by day 111 in Group 2 goats (treated on day 70). The drop in antigen levels coincided with the disappearance of trypanosomes in the peripheral circulation.

DISCUSSION

The application of polyclonal or monoclonal antibodies as antigen detecting probes for the diagnosis of parasitic infections is only a recent development. In the present study, TEA 1/23.4.6, employed for the detection of circulating trypanosomal antigens, showed fairly comparable results with those of Rae and Luckins (4) and Araujo (13). Liu et al. (6) however, using a double antibody sandwich ELISA were able to detect trypanosomal antigens from as few as 50-500 trypanosomes/well or 1-5 ng of trypanosomal membrane protein/well. This was much lower than what we could detect. Nantulya et al. (14) on the other hand, using a MAb double sandwich ELISA were able to detect as low as $1x10^5$ trypanosomes/ml or 10,000 trypanosomes per 100 ul. This latter observation is comparable to the results obtained in this study. The superior sensitivity observed in the polyclonal antibody sandwich ELISA reported by Liu et al. (6) is due to the fact that polyclonal antibodies are

directed against many different epitopes. So different antigens are detected. This leads to enhanced sensitivity. On the other hand, a MAb is directed against a single epitope. This may lead to a lower sensitivity if such a MAb is used in a double sandwich ELISA.

In this study, circulating antigens were demonstrable by day one (in Group 1 goats) and day six (in Group 2 goats) following infection and coincided with the appearance of trypanosomes in peripheral circulation. Rae and Luckins (4) found that in goats experimentally infected with T.(D). vivax, T.(T).b. brucei and T.(T).b. evansi, circulating trypanosomal antigens were detected in a sandwich ELISA within 10-40 days of infection.

Following treatment, circulating antigens dropped to pre-infection levels between day 12 and 41. Rae and Luckins (4) observed that after treatment the level of circulating trypanosomal antigens in infected rabbits dropped to pre-infection values within 7 days, while antibodies persisted longer. Liu et al.(6) also observed that in monkeys infected with T.(T).b. rhodesiense and then treated with the trypanocidal drug, melarsoprol (Mel B), antigens could not be detected by as early as day 27 after treatment and none of the sera taken after successful drug treatment (105-933 days post-treatment) showed any trace of detectable trypanosomal antigens in serum.

The observations made in this study agree with those of other workers. It is thus evident that MAbs like TEA 1/23.4.6 used in this study, can be successfully employed

for use in the diagnosis of African Trypanosomiasis as trypanosome antigen detecting probes. This test will be better than the microscopic examination of stained or concentrated blood specimens because as little as 1.25ug/ml of trypanosomal lysate antigen could be detected. Secondly, trypanosomal antigens could be detected even in the absence of detectable parasitaemia (Figures 2 and 3). Thus, the appearance of intermittent parasitaemia associated with the phenomenon of antigenic variation does not affect the test because the antigen being detected is released when the trypanosomes are lysed (10). This may explain why there was lack correlation between parasitaemia and antigen-detection as shown in Figure 2. One of the advantages of using monoclonal antibodies instead of polyclonal antibodies is that the former can be more easily standardized than the latter.

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