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ADHERENCE OF SENSITIZED TRYPANOSOMES TO PERITONEAL CELLS

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SUMMARY

Trypanosoma lewisi and *T. cruzi* adhere to peritoneal rat cells on microscopic slides, after sensitization with specific antisera and complement. Under these conditions 60 to 90% of the monocytes and macrophages have adherent trypanosomes, while most lymphocytes do not have any adherent parasites. The addition of complement enhances the reaction, but is not essential to it.

INTRODUCTION

LAVERAN & MESNIL [6] described the adherence of *Trypanosoma lewisi* to peritoneal cells of immunized rats and their ultimate engulfment. They were able to observe the complete process on slides maintained at 37°C, in peritoneal exudate of immune rats reinoculated with infected blood 30 minutes previously. A few years later, the same phenomenon was observed by MESNIL & BRIMONT [9] to occur also with other trypanosome species. They worked with African trypanosomes and specific antibody, collecting a specimen of peritoneal exudate 30 minutes after peritoneal inoculation.

A peculiar antibody, "adhesin", and complement were thought by others to be involved in this adhesion [10, 11] which was generally considered to be unrelated to protective immunity [5].

The purpose of the present paper is: a) to describe a simple technique which permits the observation and measurement of this phenomenon *"in vitro"* and b) to present initial results of experiments aiming to clarify its mechanism.

MATERIAL AND METHODS

Antisera — a) Anti - *T. lewisii* (hyperimmune rat serum): several white rats, after recovering from an initial infection with *T. lewisi*, were reinoculated with 0.5 ml of rat blood heavily infected with the same trypanosome, killed after 4 days, and their sera pooled and frozen; b) Anti — *T. lewisi* (during the course of infection): a number of white rats were inoculated with *T. lewisi* and the serum of one rat was collected daily; c) Anti — *T. cruzi* (hyperimmune horse serum). This was kindly furnished by Prof. J. Muniz, of the Instituto Oswaldo Cruz, and was obtained by hyperimmunization of a horse with culture forms of *T. cruzi*.

Peritoneal cells — 10 ml of Hanks' solution with heparin was injected into the peritoneum of adult anesthetized rats. After slight abdominal massage the liquid was withdrawn. The exudate was diluted in Turck's solution, white cells counted in a hemacytometer and their concentration adjusted to 150-200/mm^3 in Hanks' solution. Of this suspension 0.3 ml were pipetted into 12 mm x 3 mm high lucite rings fixed on microscopic slides with vaseline. These slides were incubated at 37°C for 20 minutes and rinsed in several changes of Hanks' solution.

Trypanosomes — a) *T. lewisi.* Blood trypanosomes were harvested from rats on the 2nd and 7th day of infection. Blood was

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withdrawn from the heart, defibrinated with glass beads, centrifuged at low speed for 5 minutes; trypanosomes obtained in the plasma were spun down, washed twice in saline, suspended in saline and counted in the hemacytometer, and their concentration adjusted to 10^7 cells per ml; b) *T. cruzi* — Blood trypanosomes were isolated in similar manner from mice or rats on the 10th day of infection.

Complement — Fresh rat serum or rat serum stored at -10°C was used as source of complement.

EDTA — A 0.1 M solution of ethylenediaminotetracetic acid — disodium salt, had its pH adjusted to 7.8 with NaOH. Final concentration of EDTA in tubes was 0.01M.

Reaction — The technique employed is essentially that described by LAY & NUSSENZWEIG [7]. Antisera were inactivated at 56°C, for 20 minutes. Trypanosomes washed and suspended in physiologic saline were incubated with antiserum and complement at 37°C, for 45 minutes. The total volume was 0.3 ml, final concentration of serum 1:10, final concentration of complement 1:15 and final number of trypanosomes 8 to 10 millions per ml. EDTA was added only when mentioned.

After this incubation, trypanosomes were spun down by centrifugation, resuspended in the same volume of Hanks' solution, washed once in this solution, and put on slides with lucite rings already containing adherent peritoneal cells. After another incubation at 37°C for 30 minutes, the lucite rings were removed and the slides rinsed in several changes of Hanks' solution for removal of non-adherent trypanosomes. The slides were then dipped into a solution containing 10% calf serum, 40% Hanks' solution and 50% distilled water, immediately dried under an electrical fan and stained with Leishman's stain. Uninfected rat sera and uninfected horse serum were used as negative controls.

RESULTS

The results of several experiments are summarized in Table I. Washed blood forms of *T. lewisi* obtained on the second or on the seventh day of infection did not adhere to rat peritoneal cells. If, however, these trypanosomes were treated with a hyperimmune rat anti — *T. lewisi* serum and complement, and subsequently washed, they adhered to the membranes of peritoneal rat macrophages forming "rosettes" as shown in Figs. 1-4. The reaction was immunologically specific: the same rat anti — *T. lewisi* serum did not induce the adherence of *T. cruzi* to the rat macrophages. Also, washed *T. cruzi* forms obtained at the 10th day after injection of rats and mice did not form "rosettes" when previously incubated with clean horse serum but they were formed when previously sensitized by a hyperimmune serum obtained from a horse injected many times with culture forms of *T. cruzi*. The peritoneal cells involved were mostly mononuclear cells with the morphologic characteristics of macrophages. Very seldom trypanosomes adhered to cells which had the appearance of small lymphocytes. All trypanosomes adhering to macrophages do so with their posterior end, the flagellum remaining free.

Adherence was also observed in the absence of complement, but it was clearly less pronounced. For example, several experiments were performed with *T. lewisi* anti — *T. lewisi* complexes prepared in the presence of heat-decomplemented rat serum, and the percentage of rat macrophages showing adherent trypanosomes varied between 20 and 40%. When, however, fresh rat serum was added to the complexes, 60 to 90% of the macrophages were positive.

Adherence-causing antibodies for *T. lewisi* first appear in the blood of infected animals between the 3rd and 5th day after inoculation, persist at least until the 21rst day and are present in hyperimmune serum.

Finally, we observed that the presence of EDTA in the incubation medium containing the sensitized trypanosomes and the macrophages inhibits the formation of "rosettes". This inhibition is observed with *T. lewisi* — anti — *T. lewisi* complexes prepared in the absence or in the presence of complement. When the complexes contain complement the inhibition is more striking, and the adherence to macrophages may drop from 90% to 10%.

KLOETZEL, J. & DEANE, M. P. — Adherence of sensitized trypanosomes to peritoneal cells. *Rev. Inst. Med. trop. São Paulo* 12:383-387, 1970.

TABLE I

Adherence of trypanosome-antibody-complement complexes to rat peritoneal cells

Antisera used to sensitize trypanosomes	Trypanosomes		Result of adherence test
	Species	Days of infection of donor animal	
Rat anti-lewisi			
Hyperimmune pool	*T. lewisi*	2	+
Hyperimmune pool	*T. lewisi*	7	+
Hyperimmune pool	*T. cruzi*	10	–
2nd day of infection	*T. lewisi*	2	–
2nd day of infection	*T. lewisi*	7	–
5th day of infection	*T. lewisi*	2	±
5th day of infection	*T. lewisi*	7	±
8th day of infection	*T. lewisi*	2	+
8th day of infection	*T. lewisi*	7	+
21rst day after inoculation	*T. lewisi*	2	+
21rst day after inoculation	*T. lewisi*	7	+
Horse anti-cruzi			
Hyperimmune serum	*T. cruzi*	10	+
Controls			
Normal rat serum	*T. lewisi*	2	–
Normal rat serum	*T. lewisi*	7	–
Normal horse serum	*T. cruzi*	10	–

DISCUSSION

Two classes of cytophilic antibodies have been described, γG (1,4 and γM globulin, and they have different properties. While the antigen- γG complexes bind to macrophages in the absence of divalent cations [7], the binding of antigen γM complexes is Ca^{++} dependent [8]. Our experiments show that hyperimmune rat anti- *T. lewisi* antisera contain cytophilic antibodies, as indicated by the fact that complexes of *T. lewisi* — anti- *T. lewisi* bind to the membrane of macrophages, in the absence of complement.

Complement fixation on the membrane of the trypanosomes, in the conditions of the test, does not induce appreciable lysis but renders the trypanosomes highly susceptible to engulfment by phagocytic cells. There is a remarkable increase in the adherence of trypanosomes — antibody complexes to macrophages after complement fixation. This phenomenon is similar to that observed by LAY & NUSSENZWEIG [7] using sheep red blood cells as antigen. We have also confirmed their observation that divalent cations are essential for the binding of antigen-antibody-complement complexes to macrophages, since the addition of EDTA decreases the percentage of adherent cells to an appreciable extent.

The fact that sensitized trypanosomes adhere to macrophages with their posterior end is in accordance with observations made on agglutination, originally described by LAVERAN &

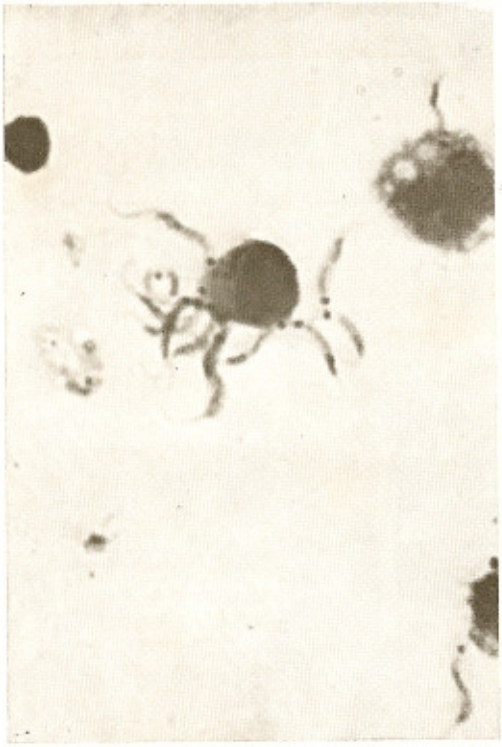

Fig. 1 — Blood forms of *T. lewisi* adhering to macrophages of the peritoneum of clean rats, after sensitization with hyperimmune anti-*lewisi* serum

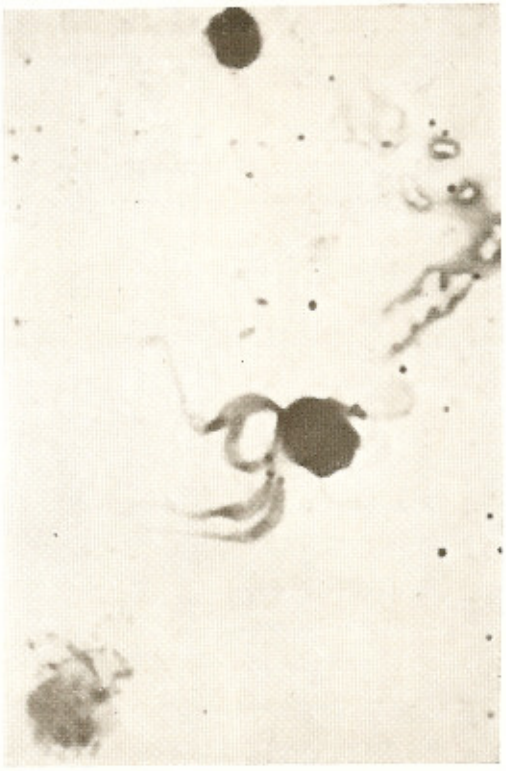

Fig. 3 — Blood forms of *T. lewisi* adhering to macrophages of the peritoneum of clean rats, after sensitization with hyperimmune anti-*lewisi* serum

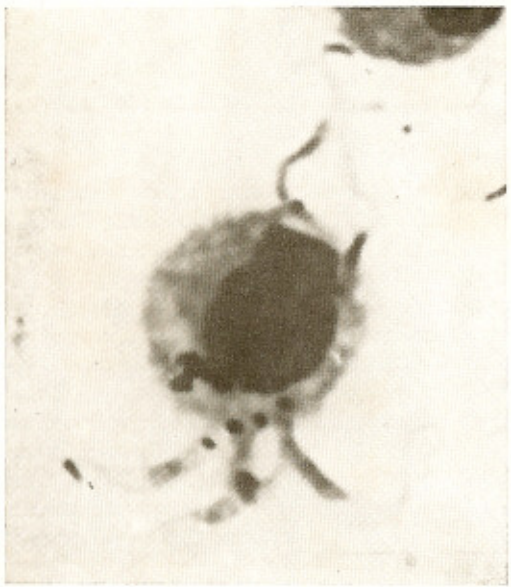

Fig. 2 — Blood forms of *T. lewisi* adhering to macrophages of the peritoneum of clean rats, after sensitization with hyperimmune anti-*lewisi* serum

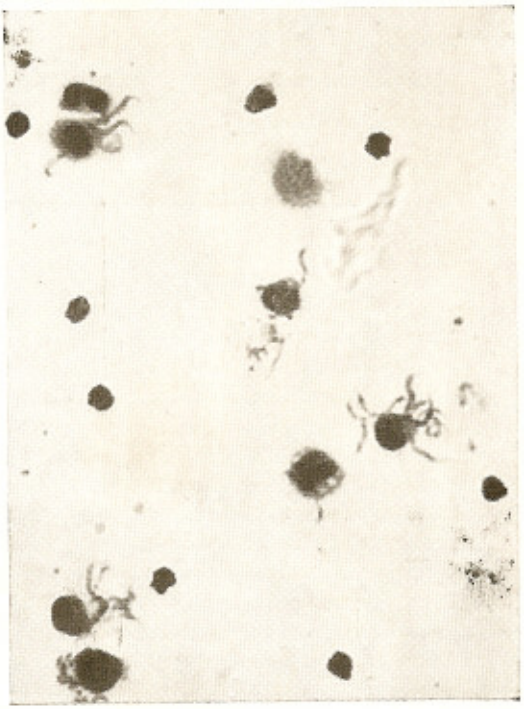

Fig. 4 — A lower magnification to show that no trypanosome is adherent to lymphocytes

MESNIL [6] for *T. lewisi*. The orientation of trypanosomes in rosettes formed by agglutination is always with the posterior end in the middle of the rosette and a free flagellum. This positioning of agglutination and adherence points to the localization of the corresponding antigen in the posterior end of trypanosomes.

The adherence was specific, and no cross-reaction between *T. lewisi* and *T. cruzi* was observed.

Although the literature on immunity in trypanosomes is extensive [13], the mechanism by which animals recover from infection, or resist reinfection is in most instances not well known. In *T. cruzi*, both humoral and cellular immunity seem to play an important role [12]. In mice immunized with *T. gambiense*, trypanosomes from homologous strains adhere to peritoneal macrophages, *in vivo* [3].

Our results again suggest that phagocytosis, as well as cytophilic antibodies play an important role in protective immunity against trypanosomes.

RESUMO

Aderência de tripanosoma sensibilizados a células de peritônio

Trypanosoma lewisi e *T. cruzi* sensibilizados com antisôro específico e complemento, aderem a células peritoneais de rato em lâminas. Nestas condições, 60 a 90% dos monócitos e macrófagos apresentam aderência de tripanosomas, enquanto a maioria dos linfócitos não têm parasitas aderentes. O complemento intensifica esta reação, porém sua presença não é indispensável.

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