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Trypanosoma evansi : in-vitro serum-dependent Phagocytosis

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Résumé

TEJERO (F.), ARISPE (M.). Trypanosoma evansi : Opsonisation in vitro. Rev. Elev. Méd. vét. Pays trop., 1984, 37 (N° spécial) : 263-269

Des expériences conduites in vitro avec une souche équine de Trypanosoma evansi ont montré que la reconnaissance et la fixation par les macrophages du péritoine du rat dépendent du type de sérum employé. Avec un sérum hétérologue, il n'y a eu aucune fixation alors que les indices ont augmenté progressivement par l'emploi d'un sérum homologue, immun et hyperimmun. Toutes les fois qu'un flagellé était phagocyté, il était invariablement détruit.

Les trypanosomes de la section Salivaria sont des parasites extracellulaires du sang. D'où l'hypothèse d'un composant sérique qui pourrait constituer le principal mécanisme de défense. Cependant il a été démontré que la phagocytose joue un rôle essentiel in vivo dans la destruction des trypanosomes africains.

Cet article rend compte de la phagocytose in vitro par des macrophages du péritoine du rat d'un variant diskinétoplastique de Trypanosoma evansi (T. venezuelense).

Mots-clés : Trypanosoma evansi - Macrophages - Rat - Phagocytose - Sérum - Vénézuela.

Summary

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In vitro experiments with an equine strain of Trypanosoma evansi have shown that recognition of and attachment by rat peritoneal macrophages are dependent on the type of serum used. With heterologous serum there was no attachment and the attachment indices increased successively with the use of homologous, immune and hyperimmune sera. Once a flagelate was ingested, it was invariably destroyed.

Key words : Trypanosoma evansi - Macrophages - Rat - Phagocytosis - Serum - Venezuela.

INTRODUCTION

Trypanosomes of the section Salivaria are extracellular blood parasites, leading to the supposition that the humoral component would be the principal mechanism of defense. However, it has been shown that phagocytosis is essential in the clearing action on african trypanosomes in vivo (3).

The present paper reports on in vitro phagocytosis by rat peritoneal macrophages of a diskinetoplastic variant of Trypanosoma evansi (T. venezuelense) (6).

MATERIALS AND METHODS

Parasites.

The strain of T. evansi used had been isolated from a case of equine "derrengadera". It has been maintained for several years by weekly passage in NMRI mice and Sprage-Dawley rats.

Blood was taken by cardiopuncture from 100-150 g male rats in 4th day of parasitemia and centrifuged in Dextran (9). The trypomastigotes were washed 3 times (1 000 x g for 15 mn at 4°C) in saline phosphate buffer plus 1 p.100 glucose (GBS), and resuspended in minimum essential medium (MEM) plus 10 p.100 tryptose phosphate broth (TPB). Cellular viability was tested with 1 p.100 trypan blue (TB), and the trypomastigotes counted in a haemocytometer.

Cell cultures

Peritoneal exudate from 100-150 g male rats was taken (11), washed 3 times (300 x g for 15 mn at 4°C) with GBS, and resuspended in MEM plus 10 p.100 TPB. Cell viability was checked with TB as above and the cells counted in a haemocytometer. Linbro plates (N° 76-058-05) with 6 wells each, bottoms covered by 22 x 22 mm coverslips, were seeded with 5 x 10^6 cells/ml suspended in 4.15 ml MEM plus 10 p.100 fetal calf serum (FCS) plus 0.1 mg/ml Gentamycin. The plates were incubated overnight at 37°C, and the supernatant was discarded and the wells washed vigorously 3 times with GBS.

Sera

Immune rat serum (12), hyperimmune rat serum (1), normal rat serum and FCS were tested. Half of the volume of each lot of serum was inactivated by heating to 56°C for 30 mn.

Interaction

For each of the 8 lots of serum, inactivated or not, 4 Linbro plates were prepared. Each well of the plates contained 4.15 ml of MEM with 10 p.100 TPB and 10 p.100 of the serum in question was seeded with 25 x 10^6 trypomastigotes of T. evansi/ml, suspended in 20 microliters GBS. After 15 mn, and every 15 mn thereafter up to 120 mn, 3 coverslips were taken from the preparation being tested. The coverslips were washed vigorously 3 times in GBS, fixed 5 mn in methanol, stained 45 mn with 10 p.100 Giemsa, and mounted in DPX. From each mounted coverslip, 500 cells were randomly selected for calculation of the attachment index (A.I.) and interiorization index (I.I.) (13). The average number of trypomastigotes adhering to the surface of the macrophage (T/M) was also calculated.

Experiments were in triplicate for each of the sera.

RESULTS

Attachment of T. evansi to the macrophages was very rapid, giving maximum A.I. values of 40.7 for inactivated hyperimmune rat serum, and 6.3 for normal homologous serum, within the first 15 mn. No recognition was shown by macrophages with FCS (fig. n°1).

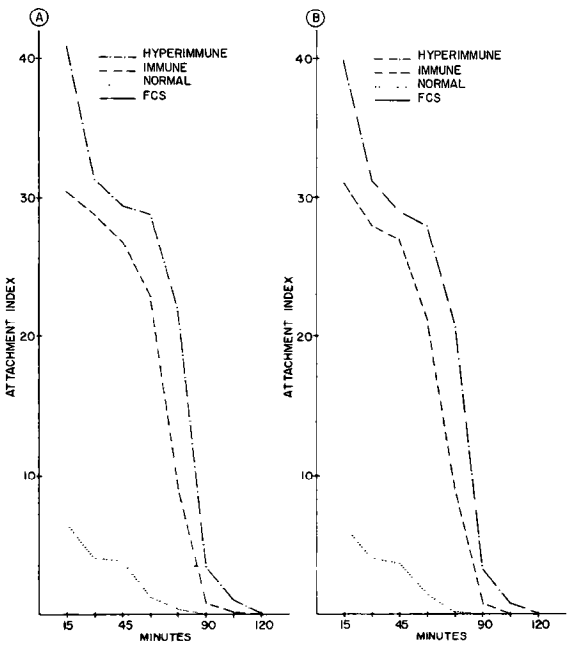

Figure n° 1. Attachment Index of Trypanosoma evansi to rat peritoneal macrophages in the presence of hyperimmune, immune, normal homologous, or fetal calf (heterologous) serum (FCS); heat inactivated (A) or not (B).

The rate of diminution of the A.I. (slope of the regression line for the observed values) indicates that the attachment process was more rapid for the higher A.I. values (table N° I).

TABLE N°I - Rate of diminution of attachment index

HOMS		IMMS		HIMS	
i	n	i	n	i	n
-0.0859	-0.0858	-0.3929	-0.3944	-0.4141	-0.4091
(-0.97)	(-0.97)	(-0.95)	(-0.96)	(-0.96)	(-0.96)

HOMS = homologous serum; IMMS = immune serum; HIMS = hyperimmune serum; i = inactivated; n = not inactivated.

The number in parentheses are the values of the correlation coefficient (r).

The highest T/M values, obtained within the first 15 mn, were between 12.78 for inactivated hyperimmune serum, and 2.88 for normal homologous serum (fig. n°2). The rate of diminution was greater for the higher values (table N°II).

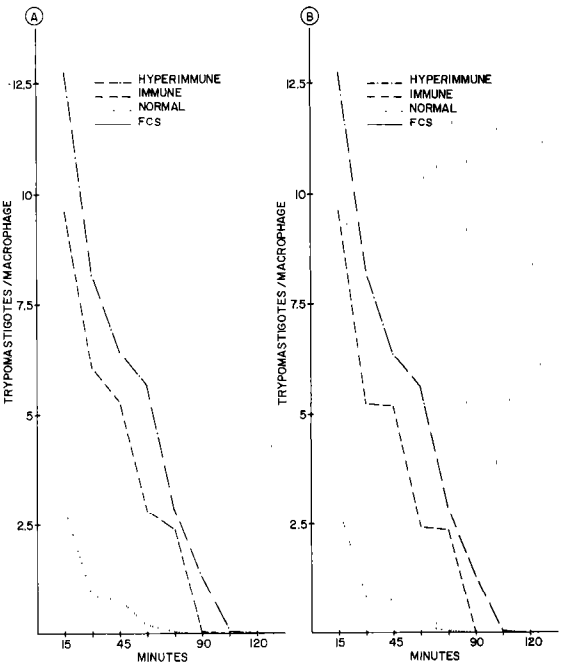

Figure n° 2. Course of Trypanosoma evansi trypomastigotes attaching to rat peritoneal macrophages cultivated with the addition of hyperimmune, immune, normal homologous, or fetal calf (heterologous) serum (FCS); heat inactivated (A) or not (B).

TABLE N°II-Rate of diminution of trypomastigotes attaching to macrophages

HOMS		IMMS		HIMS	
i	n	i	n	i	n
-0.0333	-0.0330	-0.1045	-0.1130	-0.1179	-0.1177
(-0.85)	(-0.85)	(-0.97)	(-0.95)	(-0.97)	(-0.97)

HOMS = homologous serum; IMMS = immune serum; HIMS = hyperimmune serum; i = inactivated; n = not inactivated.

The number in parentheses are the values of the correlation coefficient (r).

The highest I.I. values were obtained within the first 30 mn, for all the sera tested (fig. n° 3).

Inactivation of the sera did not significantly modify the results.

Ingested trypomastigotes were always destroyed within 30-45 mn.

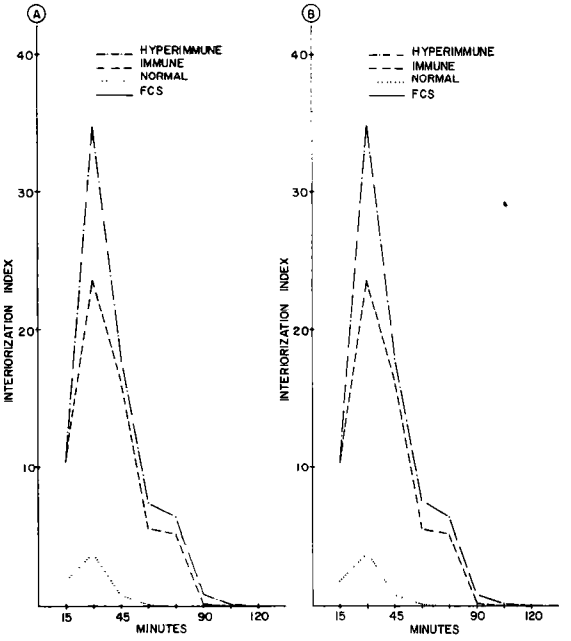

Figure n° 3. Interiorization index of Trypanosoma evansi to rat peritoneal macrophages in the presence of hyperimmune, immune, normal hologous, or fetal calf (heterologous) serum (FCS); heat inactivated (A) or not (B).

DISCUSSION

The in vitro attachment of T. evansi parasites to rat peritoneal macrophages is serum-dependent; the increase of the capacity to attach seen in the presence of immune and hyperimmune serum may be due to the presence of considerable quantities of specific antibodies. In contrast to our results, absence of attachment in the presence of homologous serum has been demonstrated in T. brucei (2, 7, 10), and in T. rhodesiense (4). In vitro phagocytosis of T. evansi even with heterologous serum has been reported (8).

The rapid extinction of the A.I. and T/M might be due to exhaustion in the culture medium of antigen-antibody complexes recognizable by the FC receptors of the macrophage.

The I.I.s' being only slightly less than the A.I.s' clearly shows the engulfing efficiency of the macrophage.

Similar values for A.I., I.I. and T/M, whether the sera were inactivated or not, suggest that complement does not participate in the interaction with T. evansi; similar results with T. gambiense have been reported (13). However, the C3b receptors on the macrophage are described as participating in the attachment of T. brucei (2, 5) and in the interiorization of T. rhodesiense (4).

The in vitro attachment of T. evansi to rat peritoneal macrophages is mediated by antibodies; the presence of immune or hyperimmune serum increases it. The interiorized parasites were destroyed.

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Resumen

TEJERO (F.), ARISPE (M.). Trypanosoma evansi : Opsonización in vitro. Rev. Elev. Méd. vét. Pays trop., 1984, 37 (N° spécial) : 263-269

Experimentos in vitro con una cepa equina de Trypanosoma evansi han mostrado que el reconocimiento y enlazamiento a macrófagos peritoneales de rata son dependientes del tipo de suero empleado. No hubo enlazamiento en presencia de suero heterólogo y los indices de enlazamiento fueron crecientes con el uso de suero homólogo, inmune e hiperinmune. Una vez ingerido el flagelado, invariablemente fué destruido.

Palabras claves : Trypanosoma evansi - Macrófagos - Fagocitosis - Suero - Venezuela.

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