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ARTÍCULO ORIGINAL

Effect of Trypanosoma lewisi *infection on the* Toxoplasma gondii *multiplication in white rat peritoneal macrophages*

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ABSTRACT

Peritoneal macrophages (PM) from normal Wistar rats were treated in vitro with peritoneal supernatant or sera, obtained from rats infected with 10^6 ***Trypanosoma lewisi*** *trypomastigotes before the infection with* ***Toxoplasma gondii*** *tachyzoites. In this experimental model,* ***Toxoplasma*** *multiplication in PM was increased, as compared to macrophages treated with supernatant or sera from control rats not infected with* ***T. lewisi****. This effect was observed only if the active supernatant or sera came from rats infected with the* ***T. lewisi*** *3 to 6 d before* ***Toxoplasma*** *inoculation. Furthermore, immunosuppressive activity was only detectable after at least 24 h incubation with the supernatant or sera. These results are in accordance with our in vivo previous studies which demonstrated a clear immunosuppressive effect of* ***T. lewisi*** *during* ***T.gondii*** *infection of the remarkably resistant Wistar rats.*

Key words*: Immunosuppression,* ***Toxoplasma gondii, Trypanosoma lewis****i, peritoneal macrophages.*

INTRODUCTION

Immunosuppressive effects in parasitic infections allow greater parasite multiplication in the final host, causing important pathology development. That is the case of filariosis, schistosomosis, trichinosis and others diseases[1,2]. More specifically in Protozoa, such as malaria infection[3] and trypanosomosis[4] immunosuppression has been reported. It has been demonstrated that the "brucei" group species exert a significant immune inhibition effect in animals and human beings[5-8]. A similar phenomenon has been observed in *Trypanosoma musculi* infections[9].

Immunosuppression can be achieved by treatment with inflammatory inhibitors such as corticosteroids[10], but this process is usually very slow. However in a recent model we have demonstrated that *T. lewisi* infections make the white rat, an animal remarkably resistant to *T. gondii*[11], as susceptible as the white mouse[12]. In this case the effect is produced only after 4 days of *T. lewisi* infection[13]. On the other hand another study showed that macrophages from *T. lewisi*

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infected rats do not present any increased multiplication of *T. gondii in vitro*[14].

The immunosuppresive effect on rat normal macrophages caused by immune supernatant and sera from *T. lewisi* infected rats is the aim of this study.

MATERIAL AND METHODS

Wistar white rats (100-150 g body weight) were obtained from Animal Care Laboratories, Hospital México, and San José, Costa Rica. The animals were infected with *T. lewisi* and served as a source of peritoneal macrophages. NGP white mice (20-25 g) were infected with *T. gondii* to obtain tachyzoites.

Tachyzoites of the RH strain of *T. gondii* obtained from infected mice and *T. lewisi* blood forms from a strain isolated from a costarican *Rattus rattus* were used throughout these studies.

Experimental model

Non-infected white rats were injected intraperitoneally with 3 ml of 0.5% sodium caseinate in 0.85% saline solution. Five days later, peritoneal macrophages were withdrawn with Minimal Essential Medium plus 10% heat-inactivated fetal calf serum and antibiotics (MEM-FCS). The cells suspended in 0.3 ml were cultured in cover slips (22 x 22 mm) incubated in 5% CO_2 at 37°C for 24 h. The cells were washed with MEM-FCS and treated with peritoneal supernatant or sera coming from *T. lewisi* infected rats. After 24 h incubation, the cells were infected with 10^6 tachyzoites of *T. gondii*. After one and 24 h incubation periods, the cells were washed, air dried, fixed in methanol and stained with Giemsa dye for 8 min. To determine the infection rate the number of intracellular tachyzoites were counted as previously described[15].

Before collection of the intraperitoneal components used in this model, rats were inoculated with 10^6 *T. lewisi* trypomastigotes. Depending on the experiment, five to twelve hours later peritoneal exudate was withdrawn as before, and centrifuged to separate the sedimented cells from the supernatant which were used together with the immune serum from *T. lewisi* infected animals.

Statistical significance was determined by using the paired T test.

RESULTS

Macrophages from non-infected rats (normal macrophages: NM) treated with supernatant from *T. lewisi* infected animals (TS) showed an increased multiplication of intracellular *Toxoplasma* tachyzoites, compared to that of NM treated with supernatant from non infected rats (normal supernatant: NS). None of the sedimented cells increased the number of tachyzoites in the phagocytes (Figure 1).

When NM were treated with supernatant from rats infected with *T. lewisi* 12 days before, there was no evidence of increasing *T. gondii* multiplication as compared with the results observed in the corresponding control (Figure 2).

Based on these results, the time of *T. lewisi* infection necessary to produce the immunosuppressive activity was investigated. Our results showed that the immunosuppressive effect started 3 days after the infection and disappeared 9 days later (Figure 3). These data also demonstrate that the best effect is found in rats that have been infected with *T. lewisi* for 3 to 6 days before.

In order to determine the optimal time of contact necessary for the immunosuppressive manifestation *in vitro*, NM were treated with TS for 1, 2, 4 or 24 h and then infected with *T. gondii. A* higher multiplication rate was found after 24 h contact (Figure 4).

To determine whether the sera from *T. lewisi*

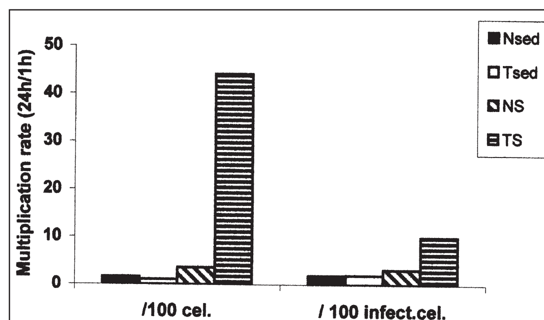

Figure 1. Multiplication rte of *T. gondii* in peritoneal macrophages treated with different peritoneal exudates componenents.
N sed = sediment from non infected rats.
T sed = sediment from *T. lewisi* infected rats.
N S = supernatant from non infected rats.
T S = supernatant from *T. lewisi* infected rats.

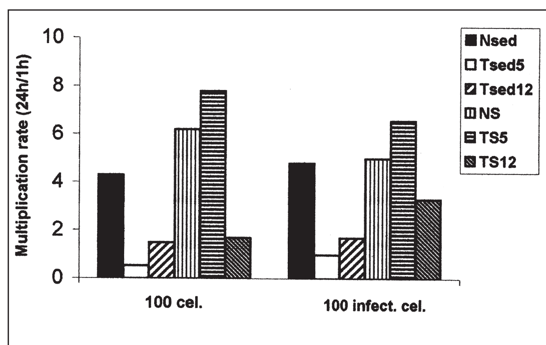

Figure 2. Multiplication rats of *T. gondii* in peritoneal macrophages treated 5 or 12 d before with different supernatant or sediments.
N sed = sediment from non infected rats.
T sed 5 = sediment from non infected rats 5 d before with *T. lewisi*.
T sed 12 = sediment from non infected rats 12 d before with *T. lewisi.*
N S = supernatant from non infected rats.
NS 5 = supernatant from rats infected 5 d before with *T. lewisi.*
NS 12 = supernatant from rats infected 12 d before with *T. lewisi.*

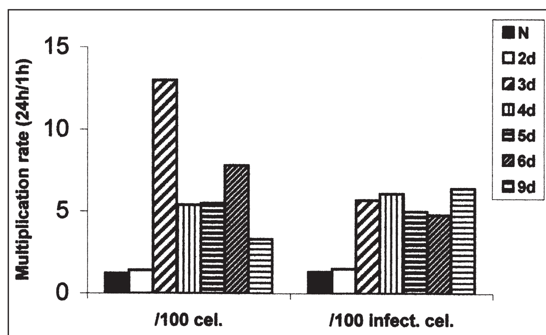

Figure 3. Multiplication rate of *T. gondii* in peritoneal macrophages treated with “active” supernatant at different periods of time.
N = normal.
d= days

infected rats produced the same effect or it was exclusively found in peritoneal supernatant, NM were treated for 24 h with sera from rats collected after 1, 2, 3, 4, 5 and 6 days of infection and then inoculated with *Toxoplasma* tachyzoites. An increased multiplication was observed in phagocytic cells treated with sera from animals infected with *T. lewisi* 3 to 6 days before (Figure 5). NM treated with sera from non-infected animals showed a normal *T. gondii* multiplication.

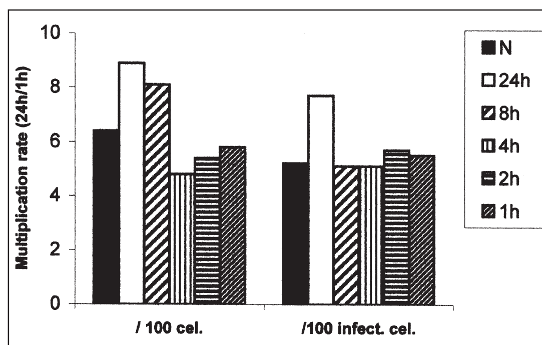

Figure 4. Multiplication rate of *T. gondii* in peritoneal macrophages treated with supernatant for different period of time (h).
N= normal
h= hours of contact

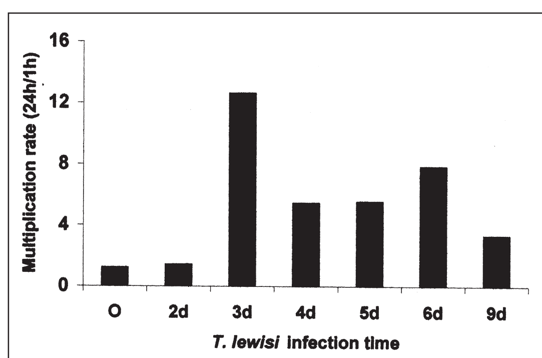

Figure 5. Multiplication rate of *T.gondii* in peritoneal macrophages treated with sera from *T.lewisi* infected rats.
N= normal
d= days of infection

DISCUSSION

Immunosuppressive activity in intracelullar organisms is a process where lymphocytes, their products, as well as phagocytic cells play an important role[16,17]. In *T. musculi,* for example, suppression of the immune defence probably depends on a direct interference on B cells[2]. These studies show, partially, an effect that could be considered similar, since the sera from *T. lewisi* infected rats induced an increase of *Toxoplasma* multiplication inside the macrophages (Figure 5). Suppression of immunity by IL-4 and IL-10 produced by spleen cells has been observed in *Trypanosoma congolense*[18] and there are similar studies with

lymphokines for other african trypanosome infections[8,19,20] and for T. *cruzi* [4]. In addition, studies dealing with gamma interferon show an apparently increase of *T. gondii* multiplication in macrophages[21]. These data agree with the results (Figures 1 and 2) because the suppressive effect was found in the peritoneal exudate supernatant where the lymphocyte products are elicited.

As in previous studies with kidney cells infected with *T. gondii*[15] the optimal contact time to demonstrate the immune effect was 24 h (Figure 4).

Gomes et al[22] reported that the glycoinositol phospholipid blocked TCD4+ y CD8+ T cells proliferation in *T. cruzi* infections which could affect the immunity in human infections with Chagas disease. Furthermore, a glycoprotein (AGG10) of this parasite inhibits the expression of IL-2 receptors chains, indicating a role for this glycoprotein in the immunosuppression as observed in the acute phase of this disease[23]. Studies in progress dealing with *T. lewisi* lysates attempt to find out if trypanosome membrane components are important in the model. In this respect we have observed that the immune effect is found in supernatants from rats infected as early as 3 days before (Figure 3). Since the animals are inoculated with 10^6 parasites, and the multiplication rate is very fast, membrane components could play an important role in the immunosuppression model reported in this paper.

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ERIKA THIERMANN ISSENSE

1916 - 2004

El viernes 11 de junio dejó de existir en el hogar donde permaneció durante sus últimos años Erika Thiermann Issense. Erika fue una extraordinaria persona, muy humana, con dotes excepcionales para la investigación y la docencia. Comenzó a trabajar en Valparaíso con el Prof. Roberto Gajardo Tobar. Posteriormente, en 1950 es contratada por el Departamento de Parasitología de la Universidad de Chile a cargo del Prof. Amador Neghme.

Sus primeros trabajos científicos los realizó sobre el *Trypanosoma cruzi* y la enfermedad de Chagas. En 1951, colabora en el primer caso de toxoplasmosis humana congénita en Chile. Desde esa fecha se hizo cargo de la sección de toxoplasmosis del Departamento de Parasitología de la Facultad de Medicina de la Universidad de Chile. Fue pionera en el estudio de la toxoplasmosis en Chile y en Latinoamérica. Su actividad científica fue muy fructífera, publicó cerca de 70 trabajos nacionales y 50 internacionales, la mayoría sobre toxoplasmosis.

El suscrito la conoció en 1957 cuando cursó el 3er. año de Medicina, posteriormente desde 1958 - 1962 pudo valorarla como ayudante alumno, y en 1962 cuando ingresó al Departamento de Parasitología, se le asignó un escritorio en el altillo de Borgoño 1470 al lado de Erika Thiermann. Desde esa fecha pude apreciar sus dotes de gran dama, y su interés por la docencia, ella me enseñó todo lo que se conocía sobre toxoplasmosis.

Su alto nivel y rigor científico, honradez a toda prueba y capacidad productiva, traspasó el país y así se hizo conocida en el extranjero. Su extraordinario fichero de los casos de toxoplasmosis nacionales e internacionales eran fuente de consulta de todos los que se dedicaban a conocer más sobre esta parasitosis.

A nombre de todos los que tuvimos el privilegio de conocerla bien, muchas gracias por todo lo que nos diste. Que el supremo hacedor te cobije en el lugar, al cual algún día todos esperamos llegar.

Dr. Werner Apt B.
Presidente
Sociedad Chilena de Parasitología