IMMUNOLOGY AND MICROBIOLOGY

Suppression of Growth and Metastasizing of T-Cell Lymphoma in Mice Infected with American Trypanosomiasis at Different Stages of Experimental Infection

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 5, pp. 542-545, May, 2004
Original article submitted January 22, 2004

Antitumor activity of *Trypanosoma cruzi* CH4 strain isolated in Mexico was studied. This parasite is not tumoritropic, but inhibits the growth and metastasizing of solid L5178Y-R lymphoma transplanted to Balb/C mice. Conditioned medium from cell cultures infected with this strain produced a cytostatic effect. Possible mechanisms of this phenomenon are discussed.

Key Words: *Trypanosoma cruzi; L5178Y-R lymphoma; antitumor activity*

Trypanosoma cruzi (*T. cruzi*), an obligate intracellular parasite, is the agent of American trypanosomiasis or Chagas disease in humans. Complex vital cycle of this parasite includes change of the host organism from insect vector to warm-blooded animal or human. Four stages of the disease are known: local inflammation at the site of insect bite, acute-phase stage developing soon after primary inoculation of the parasite into the host body and paralleled by parasitemia, asymptomatic stage lasting for 20-25 years, and chronic stage with characteristic injuries in the myocardium, gastrointestinal tract, and peripheral nerves. Long-term (virtually life-time) course of the disease associated with immunodeficiency [14] suggests simultaneous development of other diseases in the patients infected with *T. cruzi*.

Infectious agents can modify the course of some diseases. An inhibitory effect of *T. cruzi* on tumors was described [5-7,12]. Later studies made attempts at elucidation of the mechanism of this phenomenon [1-4,8,11].

We compared the effects of *T. cruzi* on the growth of transplanted lymphoma during different periods of experimental infection (acute and subacute phases) in mice and studied the relationship between the presence of the parasite and development of tumor metastases.

MATERIALS AND METHODS

The study was carried out on 8-week-old male Balb/C (H-2^d) mice highly sensitive to *T. cruzi* infection. The animals were kept under conditions of regulated temperature and day/night regimen with free access to food and water. The scheme and protocols of the experiment were designed with consideration for recommendations of ethical handling of experimental animals and were approved by Bioethics Committee of University of Colima.

Four groups of animals were used in the experiments. Group 1: *T. cruzi* acute phase+tumor. The tu-

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0007-4888/04/1375-0475

mor was transplanted on day 1 of parasitemia. The tumors were measured and histological analysis was carried out on day 23 of infection, 2 days before expected peak of parasitemia. Group 2: *T. cruzi* subacute phase+tumor. The tumors were transplanted on day 50 after infection with *T. cruzi* (late acute phase) during low parasitemia. Measurements of the tumor and its histological analysis were carried out on day 66 of infection (subacute phase). Group 3: tumor I. Tumor transplanted to intact mice, control for group 1. Group 4: tumor II. Tumor transplanted to intact mice, control for group 2.

T. cruzi strain CH4 isolated by Mario Barrero (Instituto Hideo Noguchi, Merida) from a patient with Chagas disease and cardiomegalia was used. The strain was maintained *in vitro* in LIT medium. Before the experiment mature culture containing trypomastigotes was intraperitoneally injected to mice. After 26 days experimental animals were infected with the blood of these mice containing trypomastigote forms (10^5 cells/mouse).

L5178Y-R lymphoma (H-2^d; American Type Culture Collection) was maintained in Fisher's medium (GIBCO) with 10% fetal calf serum (GIBCO) at 5% CO_2, 37°C, and 98% humidity. The medium was replaced and the culture were diluted every 3 days for maintaining the cells in log-phase. Before the experiment the cells were injected intraperitoneally to mice, which led to the formation of ascites on days 5-8. Experimental animals were subcutaneously injected with 7-day ascitic fluid (1×10^7 cell/mouse), which led to the formation of a solid tumor. The thickness of the tumors was the same, and hence, only tumor area was measured in further analyses.

Organs and tissue specimens for histological analysis were fixed in 4% formaldehyde for 3 days and then routinely processed. The sections (3 μ) were stained with hematoxylin and eosin.

B16 melanoma (American Type Culture Collection) was used in the cytotoxic test. The culture was maintained in RPMI-1640 (GIBCO) with 10% fetal calf serum (GIBCO) at 37°C, 98% humidity, and 5% CO_2. The cells were subcultured every 3 days. Log-phase melanoma cells were infected with *T. cruzi*, conditioned medium was collected after 72 h, centrifuged, and filtered. Culture medium from intact cells was also collected after 72-h incubation. For the cytotoxic test the cells were inoculated to a 24-well plate (3.5×10^4 per well). After adhesion the incubation medium was replaced with fresh incubation medium (control 1), or fresh medium with 17% culture medium from intact cell culture (control 2) or fresh medium with 17% culture medium from cell culture infected with *T. cruzi* (cytotoxic test). On day 3 of incubation the cells were treated with trypsin and their concentration was measured.

Comparative analysis was carried out using Student's *t* test ($p<0.05$).

RESULTS

Strain CH4 was not characterized in mouse models yet, and therefore at stage I we studied its biological characteristics (Fig. 1). The period between days 7 and 62 postinfection was considered as the acute phase of the disease. In our experimental model the subacute phase up to day 80 could be interpreted as early asymptomatic phase of the disease in humans.

Subcutaneous injection of lymphoblastic strain L5178Y cells to Balb/C mice led to the development of a solid tumor in 90% cases. Up to 10% animals died within 16 days. The mortality peaked between days 19 and 24, when 50% animals died. Histological analysis and measurements of the tumor size were carried out on day 16 of tumor growth, when the tumor was sufficiently large, while mortality rate remained low.

The tumor was soft, convex, with clearly defined edge, not infiltrating the adjacent tissues. On day 16 of growth the tumors implanted to animals during the acute period of infection were 1.65 times smaller than tumors in animals of the corresponding control group (Table 1). This effect was more pronounced, when tumors were implanted later and were analyzed during the subacute phase. In this case they were 2.4 times smaller than in corresponding controls. The difference in tumor size in mice with acute and subacute phases of infection was significant. It is known that the development of chronic phase of infection is associated with the appearance of bivalent antibodies to both parasite and host tissue antigens [9,10]. Presumably, tumor cells and *T. cruzi* also possess similar antigenic determinants, and hence, antibodies to the parasite

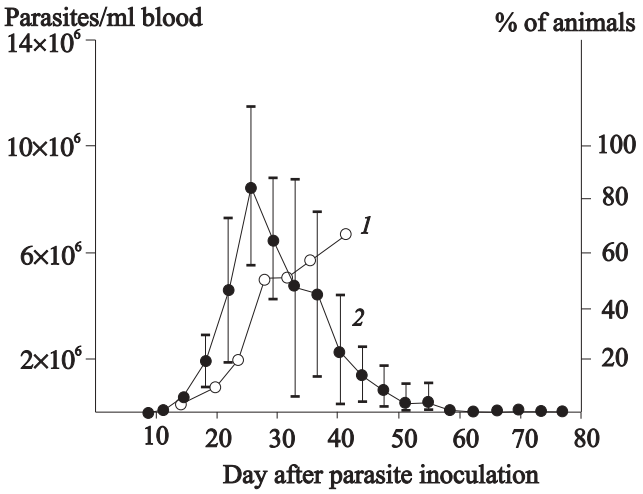

Fig. 1. Parasitemia and mortality of male Balb/C mice infected with *T. cruzi* CH4 strain. *1*) mortality; *2*) parasitemia.

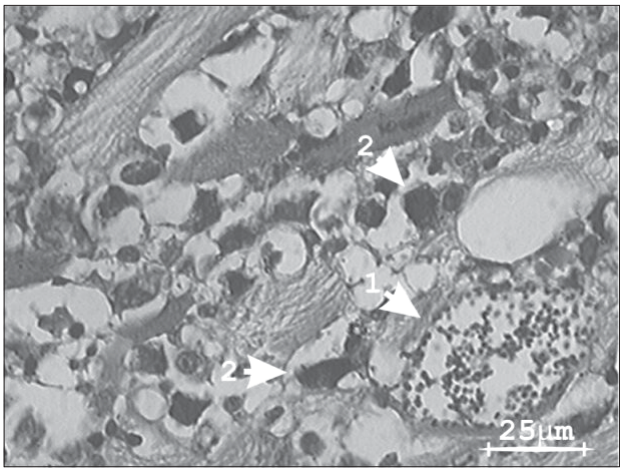

Fig. 2. Amastigotes (1) in muscle tissue of infected mice. Adjacent tumor (2) is not infected. Hematoxylin and eosin staining. Amastigotes were detected morphologically (small oval cells with kinetoplast).

bind to the tumor and suppress its growth. *T. cruzi* can act as an adjuvant and increase tumor immunogenicity. However, growth suppression in tumors transplanted on day 1 of parasitemia cannot be explained by immune mechanisms. It is unclear whether the parasite and its metabolites exert a cytotoxic or cytostatic effect and thus inhibit the tumor growth.

Professor Roskin [7] claimed that, apart from typical tissue tropism, *T. cruzi* strains can be tumoritropic and capable of infecting and damaging predominantly tumor cells. However, in our experiments the parasite was not detected in the tumor either in necrotic zones or in zones of active proliferation. No parasites were detected even in cases, when amastigotes were present in the adjacent muscles with pronounced signs of myositis (Fig. 2). Not all *T. cruzi* strains are characterized by tumoritropism (presumably, the tumor type is also significant), and tumor growth suppression probably includes several mechanisms.

Histological study on day 16 showed extensive diffuse necrotic areas occupying up to 35% of tumor volume in all tumors of the control group. This could be a result of rapid growth of the tumor bulk, while neoangiogenesis process lagged behind. Location of necrotic zones differed from that in animals infected with *T. cruzi*. There were extensive necrotic zones (up to 40% of tumor volume) at the tumor periphery clearly separated from areas with actively proliferating cells in the center. This attests to the presence of toxins modulating tumor growth and development of the adjacent tumor in infected tissue.

TABLE 1. Tumor Size in Experimental Groups

Group	Tumor area, cm^2
1st	2.758±1.216
2nd	1.768±1.090**
3rd	4.560±2.783*
4th	4.247±2.708^{+}

Note. *p=0.008, **p=0.0065 compared to group 1, ^{+}p=0.005 compared to group 2.

The cytotoxic test confirmed the presence of factors blocking cell proliferation in the culture conditioned by *T. cruzi*-infected cells. B16 melanoma cells inoculated in a concentration of 3.5×10^4 cells/well in 24-well plates reached a concentration of $1.88\pm0.25\times10^5$ cells/well 3 days after incubation (n=8). Addition of 17% cultural medium from intact cultures into the incubation medium stimulated proliferation, and after 3 days the cell concentration reached $2.24\pm0.35\times10^5$ cells/well (n=8). The effect could be due to the presence of autocrine growth factors in the conditioned medium. Addition of conditioned medium from *T. cruzi*-infected melanoma culture lead to complete inhibition of proliferation, and cell concentration remained the same as during inoculation ($3.63\pm0.6\times10^4$ cells/well; n=8).

The presence of the parasite in the blood of animals with tumors affected metastasing. In controls metastases in the liver located in the periportal area and in the parenchyma were detected in 30% cases, which is in line with previous reports [13]. The location of metastases in experimental groups was the same, but they were found in only 1 of 20 mice during the acute phase of infection, when the level of parasitemia was high (p<0.05). During the subacute phase, when parasitemia decreased, metastases were detected in 3 of 20 mice; this difference from the control was negligible. No parasites were detected in the liver.

Hence, *T. cruzi* CH4 strain exhibited antitumor activity in experiments with transplanted L5178Y-R lymphoma. The effect of tumor growth inhibition was more pronounced, if the tumor was transplanted during the late acute and analyzed during the subacute phase of infection. The mechanism of this phenomenon seems to be complex and includes production of cytotoxic/cytostatic substances and cross antibodies against both the parasite and the tumor.

The authors are grateful to Dr. Mario Barrera (Instituto Noguchi, Merida, Yucatan) for strain CH4 and to Ramon Alvarez Buylla de Aldana Foundation for financial support of the study (grant No. 150/1).

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