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In Vivo Anticancer Activity of Lysates from *Trypanosoma Cruzi* of Different Genetic Groups

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Lyzed epimastigotes of *Trypanosoma cruzi* clones P_{209-1}, $Gamba_1$, Sp_{104-1}, MAS_u, $Y_{7/1}$, MN_{12}, Cl-Brener, 86/2036, $Y_{7/2-1}$ inhibit the growth of Ehrlich adenocarcinoma in mice. The tumor decreased 1.5-3 times after 12 daily injections of lysates from 15 million epimastigotes. The protective effect progressed after the injections were discontinued and depended on the dose and lysate producer clone. Trypanosoma lysates in the studied doses were nontoxic.

Key Words: *Trypanosoma cruzi; lysate; clone; antitumor activity*

Antitumor activity of *Trypanosoma cruzi* is a known fact: 8 virulent strains causing infection in experimental animals inhibited the growth of transplanted tumor in them. *T. cruzi* strains suppressed the growth of transplanted tumors in mice. This effect depended on phenotypical characteristics of each strain [1]. Lysates of some genetically differentiated *T. cruzi* clones exhibited a cytotoxic effect on human malignant cells in experiments on cell cultures [3,4]. Activity of lysates was different: the capacity to destroy tumor cells depended on its origin from this or that clone in a genetic grouping. All these observations prompted direct *in vivo* studies of antitumor activity of lysates from biomass of *T. cruzi* of different genetic strains on mice. We studied the anticancer effects of these *T. cruzi* lysates *in vivo*.

MATERIALS AND METHODS

Nine clones belonging to different genotypical groups of *T. cruzi* species were used in the study: P_{209-1}, $Gamba_1$, Sp_{104-1} (DTU_1 group), 86/2036, CL-Brener, $Y_{7/2-1}$ (DTU_{2e} subgroup), MN_{12} (DTU_{2d} subgroup), MAS and $Y_{7/1}$ (DTU_{2-b} subgroup) — a gracious gift from Dr. Tibayrenc (Montpelier).

The clones were cultured in NNN, LIT media, and in medium developed at Moscow University in collaboration with Nutrient Media (Pitatel'nye Sredy) Company.

For obtaining lysates the cells of trypanosoma epimastigote forms at the peak of culture growth (days 14-20 after inoculation) were counted, separated by centrifugation at 3000-4000 rpm and 4°C, washed in saline, and repeatedly centrifuged; the biomass was diluted with distilled water, exposed at -70°C for at least 1 h, and then lyophilized for 5-24 h. Ready preparation was stored at -20°C.

Ehrlich adenocarcinoma cells were subcutaneously implanted to 350 outbred albino mice (0.2 ml ascitic suspension; 1×10^7 cell/mouse) in 9 experiments. Treatment with *T. cruzi* lysates from different clones was started on day 6 after transplantation. The preparations were dissolved in saline and injected subcutaneously in doses of 5 and 15 U (1 U is equal to 1 million epimastigotes) per mouse into the side contralateral to the transplantation. The

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course consisted of 12 daily injections. Controls received no lysate. The effects of the lysate were evaluated by tumor size; tumor area was measured starting from days 5-6 after transplantation at 4-6-day intervals for 50 days. The effect was evaluated by the index representing the ratio of the mean tumor area in control to the mean tumor area in experiment.

The results were processed using Statistica 6.0 software, Student's *t* test, and analysis of regressions with estimation of correlation coefficient (*r*) [2]. The differences were significant at $p<0.05$.

RESULTS

Evaluation of the mean tumor growth inhibition index showed that the lysates were little effective in a dose of 5 U/mouse: adenocarcinoma growth in experiment and control was virtually the same.

Effects of lysates in a dose of 5 U/mouse on tumor growth were detected by regression analysis [2]. In controls, the correlation *r* coefficient approached 1 (0.92±0.02) and reflected linear relationship between tumor growth and time, while in the experimental group it was 0.69±0.11 (no linear relationship).

Suppression of tumor growth was obvious in 5 experiments in which the lysate was used in a dose of 15 U/mouse (Fig. 1). The tumor was 2.5-3-fold smaller than in the control group ($p<0.05$). In some animals the tumor virtually did not grow, its size remained minimum during the entire experiment, while in others active growth of the tumor was sharply inhibited by the end of the course.

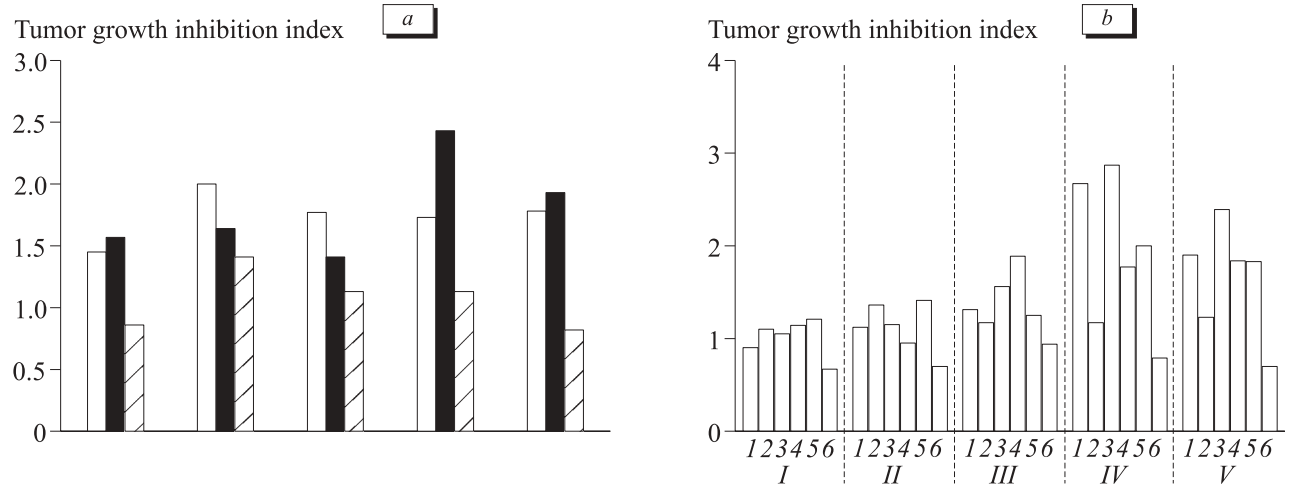

Fig. 1. Effects of *T. cruzi* lysates from DTU_1 (*a*) and DTU_2 subgroup (*b*) clones in a dose of 15 U/mouse on the growth of Ehrlich adenocarcinoma. I, II, III, IV, V: 10-day periods after adenocarcinoma transplantation. For *a*: light bars: clone P; dark bars: G; cross-hatched bars: Sp. For *b*: *1*) clone 86; *2*) CL; *3*) $Y_{7/2}$; *4*) MN; *5*) MAS; *6*) Y_7.

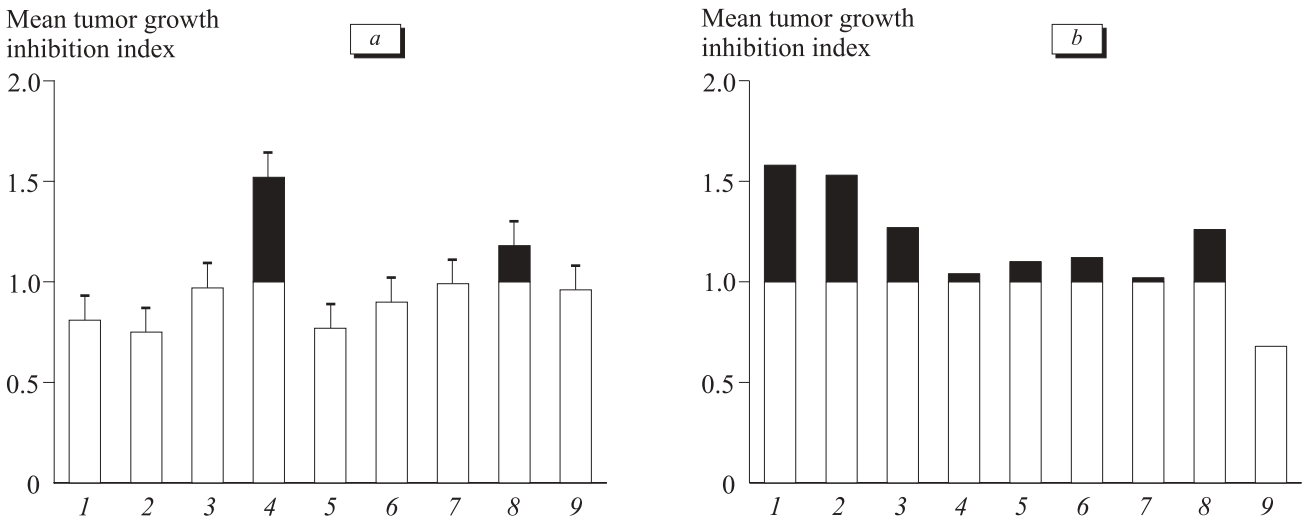

Fig. 2. Relationship between antitumor effect of different lysates in doses of 5 (*a*) and 15 U (*b*) and *T. cruzi* producer clone. *1*) P; *2*) G; *3*) Sp; *4*) 86; *5*) CL; *6*) $Y_{7/2}$; *7*) MN; *8*) MAS; *9*) Y_7. Dark segments on diagrams: significant inhibition of tumor growth.

TABLE 1. Tumor Growth Inhibition Index under the Effect of Lysates from Different Clones of *T. cruzi* in a Dose of 5 U/mouse at Different Stages of Experiment

Strain		During treatment	After treatment
P	DTU_1	0.81±0.12	0.79±0.06
G		0.75±0.70	1.01±0.11
Sp		0.97±0.05	0.68±0.03
86	DTU_{2e}	1.52±0.05	1.16±0.09
CL		0.77±0.05	0.61±0.09
$Y_{7/2}$		0.90±0.06	1.21±0.21
MN	DTU_{2d}	0.99±0.07	0.77±0.02
MAS	DTU_{2b}	1.17±0.11	1.39±0.08
Y_7		0.96±0.05	1.12±0.19

TABLE 2. Tumor Growth Inhibition Index under the Effect of Lysates from Different Clones of *T. cruzi* in a Dose of 15 U/mouse at Different Stages of Experiment

Strain		During treatment	After treatment
P	DTU_1	1.57±0.09	1.75±0.07
G		1.53±0.18	1.85±0.15
Sp		1.27±0.14	1.00±0.08
86	DTU_{2e}	1.04±0.16	1.99±0.25
CL		1.10±0.11	1.59±0.21
$Y_{7/2}$		1.12±0.05	2.39±0.26
MN	DTU_{2d}	1.02±0.07	1.81±0.07
MAS	DTU_{2b}	1.26±0.22	1.71±0.17
Y_7		0.68±0.03	0.79±0.07

Hence, the inhibition of adenocarcinoma growth depended on the *T. cruzi* clone taken for preparing the lysate. Lysates of all studied clones to a certain measure inhibited adenocarcinoma growth, the mean inhibition index varied within the range of 0.70-1.75 for different clones during the experiment (Fig. 2). The differences between the clones were more pronounced at a dose of 15 U/mouse. Lysates from clones Sp, CL, and especially Y_7 exhibited minimum activity. Weak inhibitory effects of CL and Y_7 clones on adenocarcinoma coincided with their minor effect on cancer cells in tissue culture [3,4]; for Y_7 this minor effect also coincided with low *in vivo* and *in vitro* antitumor activity of strain 3 [1], Y_7 being one of its clones. Lysates of other clones (P, G, 86, $Y_{7/2}$, MN, MAS) 1.5-1.86 times inhibited tumor growth, P, G, 86, and $Y_{7/2}$ lysates were most active (Fig. 2).

In the second part of the study the antitumor effect was analyzed during (days 6-20 after tumor transplantation) and after treatment with the preparations (days 20-45). The antitumor effect of some clones was observed during the treatment, while other clones showed a delayed effect (Tables 1, 2).

During treatment, total low activity of clone Y_7 lysate and high activity of clones P and G (at a dose of 15 U), 86 (5 U), and MAS (at both doses) were reproduced. During this period the effect was determined primarily by the direct influence on the tumor, and presumably this mechanism is essential for antitumor activities of clones P, G, 86, and MAS. However, the degree of adenocarcinoma growth inhibition during treatment with lysates correlated with their capacity to direct inhibition of cell growth in tissue cultures, though no strict correlation was observed.

The antitumor effect after lysate injection consisted of their direct influence on tumor cells and delayed effect on tumor process mediated through the immune system. Clone $Y_{7/2}$ exhibited a pronounced delayed protective effect (70% tumor growth inhibition), clone 86 showed moderate effect (34% inhibition), and clones G, MAS, MN, and Y_7 showed minor effect (16-28% inhibition). Clones P, Sp, and CL showed virtually no delayed inhibitory effect.

The capacity to direct *in vitro* effect on cancer cells [3] and *in vivo* activity of clone lysates (total and at different stages of experiment) in general corresponds to the genetic differentiation of *T. cruzi*. Clones differing by the studied sign are present in each genetic group. The direct antitumor effect of lysates is characteristic of the DTU_1 genetic group (clones P, G, Sp) and immunologically mediated effect for DTU_2 group clones (Y_7).

Hence, *T. cruzi* clones are characterized by different antitumor activity. Epimastigote lysates from clones P, G, 86, CL, $Y_{7/2}$, MN, and MAS not only suppress *in vitro* growth of malignant human cells, but also inhibit *in vivo* growth of transplanted tumor in mice. This anticancer effect is dose-dependent. The development of drugs on the basis of *T. cruzi* lysates should be carried out with consideration for clone-specific differences in the producer's antitumor activity, which is particularly important for *T. cruzi*, a flagellar eucaryotic heteroxenic parasite with pronounced clonal divergence.

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