The Study of Immunological Component in AntiTumor Effect of *Trypanosoma Cruzi*

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The experiments with passive transfer of splenocytes obtained from animals immunized with *Trypanosoma cruzi* lysate revealed the role of cell-mediated component of the immunity in the antitumor effect of *T. cruzi*. The common features of *T. cruzi* antigens and tumor-specific antigens of Ehrlich's adenocarcinoma were demonstrated. These antigens were shown to have common epitopes with mammalian mucins. The oncoprotective effect was achieved by immunization with type II and III mucins and was reproduced after passive transfer of splenocytes from immunized animals.

Key Words: *Trypanosoma cruzi; Ehrlich's adenocarcinoma; antitumor activity; immunocompetent cells*

Inhibition of growth of transplanted tumor in experimental mice after infection with *Trypanosoma cruzi* first reported in the 30s of the XX century still attracts the attention of the researches [1,3]. Progress in protist cloning made it possible to reveal strain- and clone-specific peculiarities of *T. cruzi* isolates in their antitumor effect. Specifically, this effect can be observed not only during the development of infection (including abortive one), but also after injection of trypanosomal lysates [4]. Experiments on transplanted cultures of tumor cells from mammary gland documented the cytotoxic effect of such lysates [5] thereby indicating direct action of the lysate components on tumor cells in that kind of models. However, since immune inflammation plays the key role in pathogenesis of Chagas disease, it cannot be neglected in the study of antitumor activity of *T. cruzi*.

It was established that inhibition of the growth of transplanted tumors takes place in animals preliminary immunized by trypanosomal lysate to exclude the direct effect of this parasite on the tumor [2]. In this experimental paradigm, the cytostatic effect could be mediated by non-specific stimulation of normal killers or by binding of the specifically primed antiparasitic antibodies with the tumor cells followed by their immune lysis.

Our aim was to elucidate the role of immune component in the antitumor effect of *T. cruzi*.

Literature vigorously debates on the problem of similarity between surface mucins in transplanted carcinoma cells and glycoproteins in surface structures of *T. cruzi*. Mucin-like O-glycan structures are characteristic of numerous types of human tumor cells. It is noteworthy that the mode of glycosylation and the structure of surface antigens in *T. cruzi* are similar to those in mammals [6]. Pronounced variability of the polysaccharide part of these molecules explains the ability of the parasite to escape immune control of the host organism. At the same time, this feature underlies high probability of coincidence between the immune targets in *T. cruzi* antigens and mucins of tumor cells [7].

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Based on these observations, we tried to reproduce the antitumor effect established in mice infected with *T. cruzi* by passive transfer of immunocytes from animals infected with trypanosomal lysate or mammalian mucins. We searched for trypanosomal antigens possessing common specific determinants with antigens of the transplanted tumor cells and mucins to elucidate the nature of cross-reacting antigens

MATERIALS AND METHODS

The study was carried out on transplantable tumor of the mammary gland (Ehrlich's carcinoma).

In experiment with passive transfer of immunocompetent cells, we used 100 inbred Balb mice (Kryukovo Breeding Cencer). The experiments were conducted in compliance to "Regulations to Perform Procedures with Experimental Animals" (Supplement to Order No. 755 of USSR Health Ministry on 12.08.1977).

Clone Y7/2 *T. cruzi* obtained in Laboratory UVR CNRS/IRD9996 Genetics of Infectious Diseases (Montpellier, France) was used. This clone belongs to DTU_{2e} genetic group [8]. The epimastigote forms of *Trypanosoma cruzi* were cultured in peptone-glucose medium 45A developed in M. V. Lomonosov Moscow State University and Pitatel'nye Sredy Scientific-and-Production Company (Makhachkala).

T. cruzi lysate was obtained by osmotic shock of epimastigote suspension preliminary tested by the number of parasites in 1 ml (1×10^6 trypanosomal cells were taken for one cell unit). To obtain the lysate at the peak of cell culture growth (approximately on culturing days 14-20), the epimastigote forms of *T. cruzi* were separated from the medium on the cold; thereafter, the sediment was washed with physiological saline and diluted with distilled water; 1-ml aliquots were lyophilized on a Speedwack apparatus.

The study used the following mucins: type I mucin from bovine submaxillary glands and types II and III mucins from piglet stomach (Sigma).

Group I mice were immunized with *T. cruzi* lysate in increasing doses (50-150 cell units). Groups II and III mice were immunized with type II and III mucins in the corresponding doses of 0.2 and 1.0 mg. Immunization was performed 3 times with 1-week intervals. Splenocytes were isolated on post-immunization day 10. Suspension of splenocytes was 3 times washed with Ringer's solution, diluted to a concentration of 10^6 cell/ml, and injected intravenously to intact mice. One day later, the donor mice were subcutaneously injected with Ehrlich's adenocarcinoma cells (0.2 ml or 1×10^6 cell/mouse). The control group mice were injected with splenocytes from intact donors. The blood was drawn from donor mice after immunization and from recipient mice after the end of the experiment.

Hyperimmune serum against *T. cruzi* was prepared for immunochemical assay by immunization of rabbit with the use of Freund's adjuvant followed by intravenous injections of the lysate in increasing concentrations from 200 to 1600 cell units at 10-day intervals. Antitrypanosomal serum (ATS) was decomplementized. To prevent the formation of immune complex of ATS with normal tissue antigens, it was preliminary sorbed with homogenates of parenchymatous organs from intact mice (liver, spleen, kidney, and heart). Moreover, it was additionally sorbed by the medium for culturing *T. cruzi,* thereafter this serum was used in the reaction of indirect immunofluorescence.

The presence of cross-antigens was detected by luminescence quenching after treatment of ATS with tumor cells.

To determine immunogenicity of mucins, the antigenic diagnosticums were prepared from sheep erythrocytes loaded with types I, II, or III mucins, respectively.

Similarity of antigens in Ehrlich's adenocarcinoma and mucins was tested in the reaction of inhibition of passive hemagglutination. To this end, serum was preliminary sorbed by Ehrlich's adencarcinoma cells or normal mammary gland tissue from lactating mouse. The serum was titrated with the corresponding erythrocytic diagnosticum. The presence of cross-antigens was indicated by a decrease in serum titer after its sorption by no less than two dilutions.

The data were processed statistically with Statistica 6.0 software.

RESULTS

We previously showed that among 9 clones of *T. cruzi* examined *in vitro*, Y7/2 clone demonstrated moderate cytotoxic effect against breast cancer cells [2]. However, trypanosomal lysate of this clone was most efficient in inhibition of the growth of Ehrlich's adenocarcinoma *in vivo* when it was injected before tumor transplantation. The mice with transplanted tumor had low level of antitrypanosomal antibodies [4], which suggests the involvement of the cell-mediated instead of humoral component of the immune response in the antitumor action of trypanosomal lysate.

It was shown that the tumor growth is significantly decelerated in mice injected with splenocytes from animals immunized with trypanosomal lysate

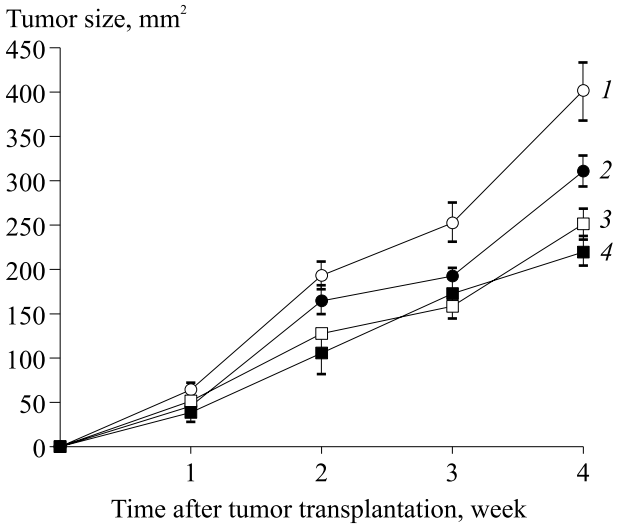

Fig. 1. Effect of splenocytes obtained from animals immunized with mammalian mucins and lysate of *T. cruzi* on the size of Ehrlich's adenocarcinoma. *1*) control; *2*) type II mucin; *3*) type III mucin; *4*) lysate of *T. cruzi*.

(Fig. 1). The difference in tumor size in the control and experimental groups became significant as early as 2 week after transfer. There were no antibodies against *T. cruzi* in the sera of experimental and control mice. The inhibition of tumor growth in these experiments was probably caused by passively transferred immunocompetent cells targeted against adenocarcinoma cells. This can be true only in case, when the antigen structure of *T. cruzi* exhibits common epitopes with tumor markers. Since mucin-like structures similar to glycosylated antigens of *T. cruzi* are the tumor markers in various carcinomas [7], it cannot be excluded that this phenomenon underlies the antitumor effect observed after immunization of the animals with trypanosomal lysate. These common features in antigen structure can be observed in certain types of mucins produced by epitheliocytes in normal tissues. Logically, it can be hypothesized that immunization of the animals with certain types of mucins or trypanosomal lysate could induce production of the effector cells that inhibit tumor growth. To test this hypothesis, we injected immunocompetent cells from mice immunized with mucins of three types to the experimental mice, and after the following transfer of the tumor, evaluated the rate of its growth in comparison with that in control animals.

In preliminary experiments, we tested immunogenicity of each mucin type. In identical immunization protocol, type I mucin induced production of antibodies with titer not higher than 1:4, while the types II and III mucins yielded antibodies with a titer of 1:64. In the following experiments, we used only these two latter mucins.

In experimental mice receiving splenocytes from the mice immunized with types II or III mucins and thereafter inoculated with adenocarcinoma, the transferred immunocompetent cells inhibited the tumor growth. The antitumor effect of type III mucin was the greatest and did not significantly differ from that of trypanosomal lysate (Fig. 1).

The data obtained with the reaction of passive hemagglutination attest to ability of ATS to agglutinate erythrocytes loaded with types II and III mucins to the titer of 1:16, which indicates the presence of antigen determinants in these mucins identical to those of the surface antigens of *T. cruzi*. At the same time, cells of the solid tumor inhibited this reaction.

Similarity of surface antigens of *T. cruzi* and those of Ehrlich's adenocarcinoma cells was confirmed by the reaction of indirect immunofluorescence. Pronounced fluorescence was detected on the surface of trypanosomal and tumor cells treated with ATS on the fixed preparations and on the live cells *in vitro*. In the latter case, non-fixed trypanosomal cells retained motility. ATS sorbed by tissue homogenates from intact mice remained active. The capacity of ATS to interact with trypanosomal and tumor cells was preserved after its sorption by the medium conditioned by trypanosomal cells and by tissues of parenchymatous organs of healthy mice. Luminescence of the surface of tumor cells was quenched after ATS was sorbed by tumor cells. At the same time, the intensity of luminescence of trypanosomal cells decreased significantly, but did not disappear.

Thus, it can be concluded that *T. cruzi* exhibits antigens similar to tumor markers of Ehrlich's adenocarcinoma. These structures include determinants common to those in mammalian mucins. The antitumor effect of trypanosomal lysate can be realized via cell-mediated component of immune reactions.

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