Genetic Heterogeneity of *Trypanosoma cruzi* and Its Direct Anticancer Effect in Cultured Human Tumor Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 1, pp. 103-106, January, 2003
Original article submitted October 25, 2002

The direct inhibitory effect of *Trypanosoma cruzi* epimastigote lysates on *in vitro* cultured human breast cancer MCF-7 cells differs in various genetic groups and cloned subgroups of Trypanosoma.

Key Words: *Trypanosoma cruzi; anticancer activity; lysate; epimastigotes; kinetoplast DNA*

It was shown that anticancer activity of *Trypanosoma cruzi* is a species characteristic of this protozoon. However, this activity varies in Trypanosoma strains differing by biological characteristics. Byotypes of *T. cruzi* are characterized by different capacity to metacyclogenesis, pathogenicity, virulence, tissue tropism [7], and interaction with host T lymphocytes and macrophages [6] and some agglutinins. They differ also by the presence of surface polysaccharides [10], rod antigens, protein, RNA and DNA content, composition of cytochromes, and neuraminidase activity.

The *T. cruzi* species is characterized by high degree of genetic structuredness. The strains differ significantly by the nuclear and kinetoplast genome: number of chromosomes, their size, gene repertoire, total cell DNA, composition of nuclear and kinetoplast DNA bases [5], maxi- and miniannular DNA molecules. By many genome markers the *T. cruzi* species can be clearly divided into 4 groups of strains.

Additional data on the intra-species genotypical variability of *T. cruzi* were obtained by enzyme immunoassay, which showed correlation of phenotype with genotype [2,4,11]. Analysis of these data helped create a new classification of *T. cruzi* strains, approved and adopted by the International Workshop on Chagas Disease in 1999.

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The differences between *T. cruzi* strains by isoenzymes, polymorphous loci, number of alleles per locus, and degree of heterozygosity sometimes surpass the common level of intraspecies differences and we can speak about several subspecies (*cruzi*-1, *cruzi*-2, and *cruzi*-3). However, in general, this genetic variety can be described as several main groups with predominance of some of the major genotypes 19, 20, 32, and 39, most prevalent among *T. cruzi*.

This approach allows us to divide *T. cruzi* into 2 main discrete typing units (DTU). DTU_1 strains possess type I zymodem (enzyme complex) with predominate of genotypes 19 and 20, the strains are highly prevalent, circulate in natural and synantropic foci (actively circulating in humans), are highly virulent, transmissible, active in metacyclogenesis, are well cultured, and little sensitive to the main chemical drugs. DTU_2 strains are less homogeneous, possess types II and III zymodem, and are subdivided into 5 subgroups with predominance of different genotypes: 27-29 (subgroup 2a), 30-33 (subgroup 2b), 35-36 (subgroup 2c), 39 (subgroup 2d) or 43 (subgroup 2e); two latter genotypes are considered to be hybrid.

The most important advantage of this classification of *T. cruzi* strains is that it is based on coinciding results of immunological method, enzyme immunoassay, and RAPD analysis (amplification of polymorphic DNA) and correlation of genetic markers with phenotypical (including histotropic) properties of the

0007-4888/03/1351-089$25.00

parasite [9]. The relationship between anticancer activity of *T. cruzi* strains and their DTU appurtenance has never been investigated.

However the differentiation of *T. cruzi* species is deeper than just the strain structure. Strains are heterogeneous and consist of a set of clones differing by many signs, including the genotypical signs: virulence, invasive activity, sensitivity to chemical drugs, capacity to metacyclogenesis, synthesis of surface antigens, including with molecular weight of 72 kDa, isoenzymes, and neuraminidase. The data on the variants of nucleotide composition and set of kinetoplast DNA miniannular molecule classes are particularly interesting in this aspect. Characterization of miniannular kinetoplast DNA is even considered as the marker of *T. cruzi* clone specificity.

Analysis of anticancer activity of different strains of *T. cruzi* was carried out at the Laboratory of Infectious Diseases Genetics in Montpelier (Head Dr. M. Tibayrenc) [1]. The species-specific status of this sign in *T. cruzi* was confirmed. All 16 tested clones belonging to different genetic groups induced death of cultured human breast cancer cells.

We compared the direct inhibitory effect of lysates of *T. cruzi* belonging to different genetic groups and subgroups on cultured human cancer cells.

MATERIALS AND METHODS

Sixteen cloned *T. cruzi* strains genetically characterized by 22 locuses by multilocus electrophoresis were obtained from UVR CNRS/IRD 9996 Laboratory "Genetics of Infectious Diseases" (Montpelier) [1,13]. These strains belong to the main genetic groups and subgroups of the species, their geographic origin is different and they were isolated from different hosts [1]. The genotype of every strain was regularly verified in the course of the study.

Epimastigote trypanosome forms were cultured in LIT (Liver Infusion Tryptose) medium. Epimastigote lysates were prepared by 3-fold freezing (-80°C) and thawing (45°C) of trypanosome mass precipitated by centrifugation. The lysates were used on the day of preparation. The lysate doses were expressed in arbitrary units; 10^6 Trypanosoma cells/ml medium was taken as one arb. unit.

The effect of epimastigote lysates on human breast cancer cells was studied in MCF-7 culture. MCF-10A cell culture (mammary gland epitheliocytes) served as the control. These cells are not tumorigenic for immunosuppressed mice, but form colonies in semisolid medium.

Cell cultures were maintained in F-12/DMEM. All cultures formed a monolayer adhering to the glass.

All lots of *T. cruzi* lysates were incubated with cultured cancer cells for 12-16 h. The lysates were used in 3 doses: maximum (500), medium (250), and minimum (125 arb. Units).

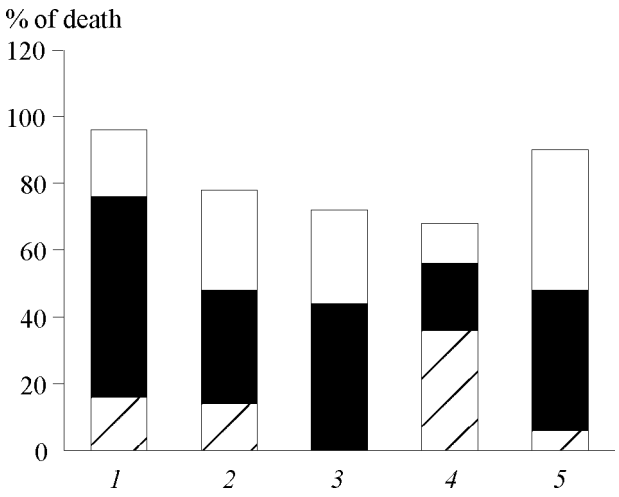

Fig. 1. Inhibition of human breast cancer cells MCF-7 by *T. cruzi* epimastigote lysates. Genetic groups and subgroups: *1*) DTU_1, *2*) DTU_2, *3*) DTU_{2b}, *4*) DTU_{2e}, *5*) DTU_{2d}. Here and in Fig. 2: light part of the bar corresponds to lysate dose of 500 arb. units, dark part to 250 arb. units, and cross-hatched part to 125 arb. units.

After incubation with *T. cruzi* lysates monolayer cultures were washed with normal saline and treated with trypsin. Anticancer effect of *T. cruzi* was evaluated after adding Trypan blue by counting the number of cells still adhering to the glass in comparison with the control.

The results were statistically processed using Student's *t* test.

RESULTS

The capacity of *T. cruzi* lysates to inhibit human cancer cells was different in different *T. cruzi* clones and correlated primarily with their appurtenance to one of the two DTU. DTU_1 clones were significantly more active than DTU_2 clones: in doses of 250 and 500 arb. units the former caused death of 76-95% cells, while the latter inhibited only 48-77% cells (Fig. 1).

Using a set of clones we found that DTU_2 group, heterogeneous by many signs, was heterogeneous by the anticancer potential of the corresponding clones. The activity of DTU_{2d} clones was similar to that of DTU_1 clones, DTU_{2b} clones showed the lowest anticancer activity, while DTU_{2e} clones showed an indirect relationship between anticancer effect and the dose.

Clone analysis showed that anticancer activity of *T. cruzi* was associated with a certain discrete genotype of the species. Since each of the studied clone groups is characterized by a certain genotypical marker, we conclude that anticancer potential of *T. cruzi* decreases in the following series of its genotypes: 19/20> 39>43>32>33. Therefore, the highest anticancer ca-

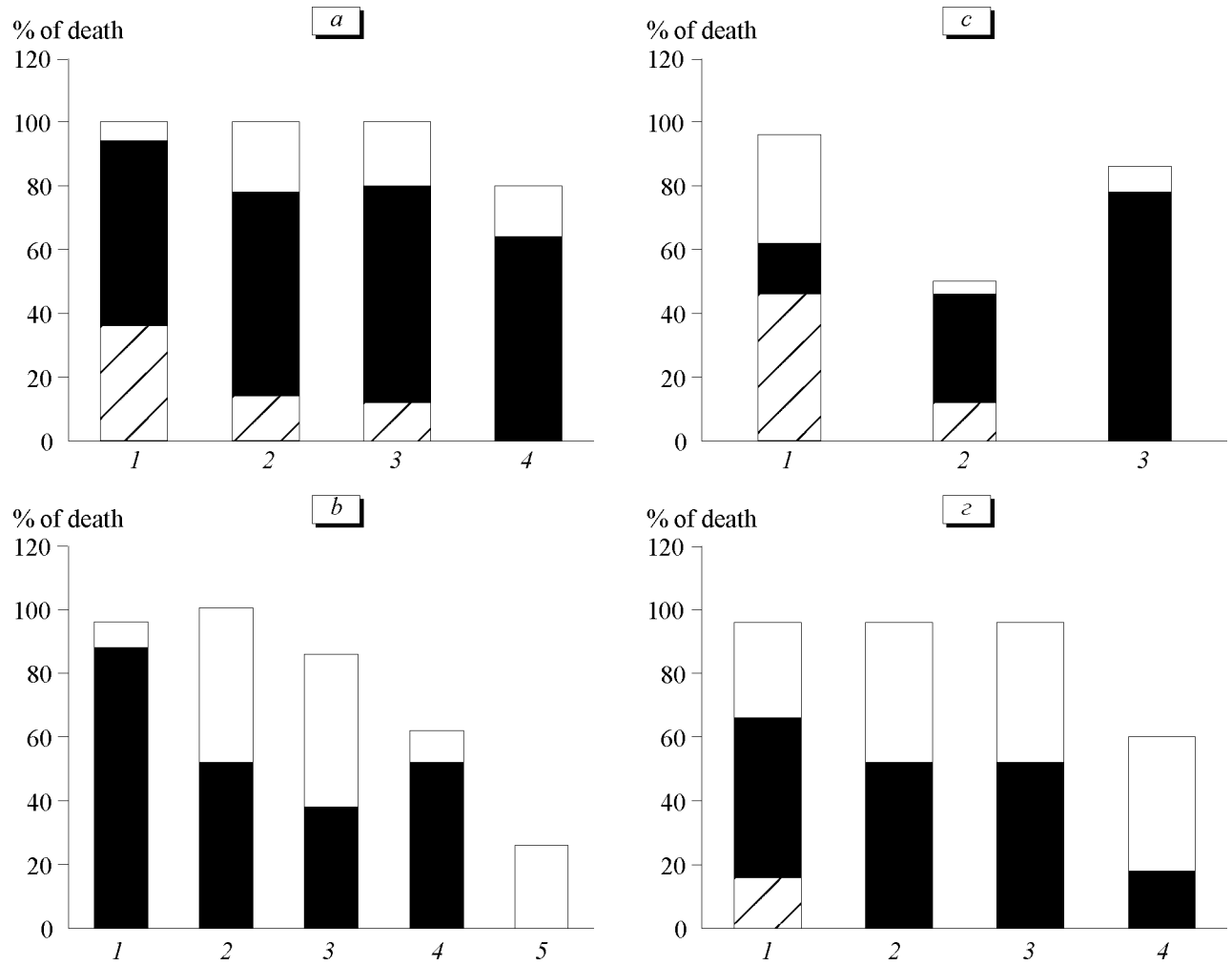

Fig. 2. Inhibition of human breast cancer cells MCF-7 by epimastigote lysates of different *T. cruzi* genotypes and DTU_1 strains (*a*) and subgroups DTU_{2b} (*b*), DTU_{2e} (*c*), and DTU_{2d} (*d*). *a*: *1*) 20 p209; *2*) 19 Gamba; *3*) 19 Sp104; *4*) 19 Cutia; *b*: *1*) 32 MAS; *2*) Y-7; *3*) 32 Tu-108; *4*) 33 CBB; *5*) 32 JVV; *c*: *1*) 43 86/2036; *2*) Cl-Br; *3*) J72; *d*: *1*) 39 MN; *2*) 39 NR; *3*) 39 Bug; *4*) 39 SO3.

pacity correlates primarily with genotype 19/20 and other signs determined by it: high prevalence in the area, high virulence, active metacyclogenesis, type I zymodem, and capacity to *in vitro* growth.

Combination capacity and intactness of the genome and particularly of glycerophosphatysomerase genes are essential for sufficient anticancer activity of *T. cruzi*. Among active genotypes of DTU_2 group are primarily genotypes 39 and 43 (DTU_{2d} and DTU_{2e}), which are particularly multilocus and possess signs of hybrids, while the least active DTU_{2b} clones are characterized by reduced genome, absence (deletions) of 700-900 b. p. fragments, and variable electrophoretic mobility of glycerophosphate isomerase [2-4,8,12]. It was found that anticancer activity correlated with more specific and fine characteristics of *T. cruzi* genome than those underlying the species differentiation into above-mentioned groups and subgroups of strains. Anticancer activity of *T. cruzi* varied within a wide range even for each studied genotype (Fig. 2). One of 3-5 clones of each category sharply differed from the rest clones by its low activity: Cutia in DTU_1 group, YVV in DTU_{2b} group, Y7-2 in DTU_{2c} subgroup, SO3 in DTU_{2d} subgroup.

The mechanism of trypanosomatide variability is still unclear, but the majority of scientists consider that kinetoplast DNA and its miniannular molecules play the key role in this phenomenon. It is very tempting to detect a correlation of the studied phenomenon with variability of these DNA-containing structures, but, despite the persuasive and deep relationships between anticancer activity of *T. cruzi* and its intra-species genetic variety, they just demonstrate the dependence of this capacity on *T. cruzi*, while the chemical nature of the active principle remains the object of further research.

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