

EFFECT OF INTERFERON ON THE DEVELOPMENT OF *TRYPANOSOMA CRUZI* IN TISSUE CULTURE "VERO" CELLS

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Results are presented on the effects of interferon on the intracellular stages of T. cruzi in tissue culture "Vero" cells. Interferon was obtained by infecting monolayers of human amniotic cells with inactivated Newcastle disease virus. Interferon has not affected the cell infection by T. cruzi culture infective stages and neither has it prevented the transformation of amastigote into trypomastigote stages.

Interferon and its inducers exert an effect on some intracellular protozoa infections such as sporozoite-induced rodent malaria (Jahiel et al., 1968), *Toxoplasma*-infected "L" cell monolayers (Remington & Merigan, 1968) and *Eimeria* grown in chick kidney cells (Fayer & Baron, 1971). The existence of an inhibitory effect of interferon on the intracellular development of *T. cruzi* might, on the other hand, influence the vertebrate host resistance since release of an interferon-like substance has been described in experimentally infected animals (Rytel & Marsden, 1970). Interferon-inducers were apparently unable to affect *T. cruzi* in tissue culture and infected animals (Kumar et al., 1971; Martinez-Silva et al., 1970). This paper studies the effects of interferon from human origin on *T. cruzi* intracellular stages in "Vero" cell monolayers.

MATERIAL AND METHODS

The following *T. cruzi* strains have been studied: Y (Pereira da Silva & Nussenzweig, 1953), CL (Brener & Chiari, 1963) and Gilmar (G) isolated in 1974 from an acute case. The methods used for the cultivation of the parasites had been described by Brener et al. (1976).

Interferon was obtained by infecting monolayers of primary human amniotic cells with Newcastle disease virus inactivated by ultraviolet light (NDV-UV), collecting the fluids after 24 or 72 h and submitting them to dialysis against pH 2 and pH 7. Titration of interferon was performed by adding dilutions to "Vero" cells and then challenging them after 24 h with about 100 TDC₅₀ of vesicular stomatitis virus (VSV). Comparative

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Supported by the National Research Council.

Received for publication on February 1st, 1980.

assays with the international standard for human interferon (research Standard B 69/19) were done and titers are expressed in international units.

For the evaluation of protective effects against *T. cruzi*, about 370 units of interferon/ml diluted in 199 medium *plus* 1% sheep serum were added to screwcap tubes containing "Vero" cells growing in flying cover-slips at 37°C. After 24 h the medium was removed, the cells washed with Hanks solution and then infected with about $1.5 - 2.0 \times 10^5$ metacyclic trypomastigotes from the cultures suspended in 1.0 ml of maintenance medium, as described by Brener et al. (1976). After further 24 h of incubation, the remaining flagellates were removed by washing with Hank's solution; medium containing 370 units/ml of interferon was then added. Control tubes with cells from the same batch were submitted to the above described procedures using, however, medium without interferon. At least five treated and control tubes were used in each experiment. On the 5th day of infection the coverslips were removed and stained according to Brener et al. (1976). The percentages of amastigote — and trypomastigote-harboring cells were determined by examining at least 300 unselected parasitized cells from each preparation under high magnification (X 1,000). Any cell presenting at least one trypomastigote was included among the trypomastigote-harboring cells since this was considered as representative of an ongoing differentiation process towards infective stages. The percentage of infected cells was estimated by determining the number of infected and uninfected cells in 50 random microscopical fields (X 1,000).

RESULTS

Table I shows the results obtained with the several experiments. Interferon is apparently not affecting the uptake of the infective stages by the "Vero" cells as demonstrated by the percentage of infected cells in treated and control tubes. On the other hand, interferon is also not preventing the transformation of amastigote into trypomastigote stages with the different strains, which readily occurs in both control and treated cells. The experiment with CL strain has been performed at 33°C because its intracellular differentiation is inhibited at 37°C (Brener et al., 1976).

TABLE I

Effect of interferon on the percentage of infected cells and percentage of trypomastigote-harboring cells in "Vero" cells infected with *T. cruzi* culture forms.

<i>T. cruzi</i> strains	<i>Infected cells (%)</i>		<i>Cells with trypomastigotes (%)</i>	
	<i>Controls</i>	<i>Interferon</i>	<i>Controls</i>	<i>Interferon</i>
Y	15.7 ± 5.0 ^a	17.3 ± 5.3 ^a	38.6 ^c	24.3 ^c
G	6.3	9.3	8.3	16.1
G	2.5	2.3	9.8	13.2
CL ^b	17.3	26.3	14.6	16.2

^a Mean of 3 experiments, $p > 10\%$, Student test

^b Experiments performed at 33°C

^c Results of one experiment at the 7th day of infection

DISCUSSION

Our data show that, conversely to what occurs with other obligatory intracellular protozoa, *T. cruzi* development is not inhibited by high doses of interferon in tissue culture. Kumar et al. (1971) had already demonstrated that the interferon stimulator polyinosinic-polycytidilic acid (Poly I:C) enhanced the parasitemia and decreased the survival time of treated mice challenged with *T. cruzi*. Similar results had been reported by Martinez-Silva et al. (1970) with the same synthetic polynucleotide in experimentally infected animals. The intracellular multiplication of *T. cruzi* in cell monolayers treated with Poly I:C was unaffected.

Our data show that *T. cruzi* intracellular stages are also not affected *in vitro* by the direct action of interferon from human origin. The dosage of interferon used in the experiments was rather high, since it displayed a strong anti-viral effect in the "Vero" cells, inhibiting more than 99,99% of VSV growth in a single cycle.

Eimeria tenella displayed a 33% growth inhibition in chicken fibroblasts when exposed to an interferon concentration which inhibits in 90% the growth of Sindbis virus (Fayer & Baron, 1971). Since this virus presents the same sensitivity than VSV in chicken cells, our data strongly suggest that the non-susceptibility of *T. cruzi* to interferon is an inherent characteristic of the parasite.

The variability in the percentages of infected "Vero" cells observed in this paper with different *T. cruzi* strains is a rather common phenomenon. Previous experiments (Bertelli et al., 1977) demonstrated that cell infection rates with *T. cruzi* culture forms depend on many factors such as the parasite strain, number of culture passages, temperature, etc. The consistent infection rates observed with the Y strain were obtained in experiments performed at short periods of time and before significant changes occurred in the culture. Nevertheless, despite the variable results it is quite clear that interferon does not hinder *T. cruzi* cell infection. A possibility exists, however, that *T. cruzi* could be sensitive to immune interferon (type II interferon) since these studies were performed with the type I interferon.

The advantages of using interferon instead of inducers is that nonspecific effects, such as the described non-interferon protection against *Toxoplasma* induced by Poly I:C in L-cells (Schmunis et al., 1973), can be prevented. Interferon is not playing, apparently, an important role in the resistance to *T. cruzi*. On the contrary, it is likely that this substance may exacerbate experimental infections by an immunosuppressive effect as suggested by Clinton et al. (1975) based on Chester et al. (1973) findings describing suppression of mice antibody-producing spleen cells by interferon.

RESUMO

Interferon obtido através da infecção de células amnióticas humanas por vírus inativado da doença de Newcastle foi incapaz de influir sobre a infectividade de formas de cultura do *T. cruzi* para células "Vero" de cultura de tecido. A transformação amastigota-tripomastigota também não foi afectada pelo interferon.

ACKNOWLEDGMENTS

The Authors thank João Rodrigues dos Santos, Maria Lúcia Trindade and Bernadete de Jesus Martins for excellent technical assistance.

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