Vol. 187, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1992 Pages 108-113

# Trypanosoma cruzi: Inhibition of Metacyclogenesis by Mannose

M.A. Barbieri*, E.M. Lammel,** , E.L.D. Isola**, and F. Bertini*,[1]

*Instituto de Histología y Embriología, Facultad de Ciencias Médicas Universidad Nacional de Cuyo, Mendoza and **Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Argentina

Received June 2, 1992

Metacyclogenesis of Trypanosoma cruzi epimastigotes was evaluated in a medium supplemented with Triatoma infestans intestinal homogenate in the presence of sugars and derivates as are mannose, galactose, fucose, N-acetylglucosamine, mannose 6-P, and fructose 1,6-P at a concentration of 25 mM. Only mannose significatively inhibited metacyclogenesis. Sodium metaperiodate and trypsin treatment of the intestinal homogenate also inhibited differentiation. In our opinion there exists a proteinic factor in the intestin of the vector that promotes metacyclogenesis and is incorporated by the parasite. Treatment of the intestinal homogenate with alkaline phosphatase had no effect. Instead, high ionic strength in the medium (0.4 M NaCl) strongly inhibited metacyclogenesis indicating that, in these conditions, the possible binding of the differentiation factor to the parasite surface was inhibited. 

It has been demonstrated that extracts of insect vector stimulate differentiation of Trypanosoma cruzi epimastigotes to metacyclic forms (1,2). Isola et al. (3) showed that the addition of a chromatographic fraction obtained from intestinal homogenate of Triatoma infestans has a metacyclogenic activity equivalent to that of the whole homogenate. This fraction, analyzed by gel electrophoresis, revealed the presence of five major protein bands with a molecular weight varying from 16 to 75 KD. Preliminary experiments (Isola, unpublished observations) showed that treatment of the fraction with trypsin abolished morphogenesis stimulation. Thus, it is possible that one (or more) proteinic factor present in the intestinal homogenate is involved in the

[1]To whom correspondence should be addressed at Inst. Histología y Embriología, Casilla de Correo 56, Mendoza 5500, Argentina.

0006-291X/92 $4.00

differentiation process of the parasite. It is well known that in other cells differentiation factors are recognized by receptors located in the plasmalemma (4). Lysosomal enzymes are not only carried in the cytoplasm, but also recaptured from the external environment by the cell, due to receptors located in the plasmalemma that recognize the hexose derivative mannose 6-phosphate (5).

In view of these findings, we figure out that the differentiation of epimastigotes to the metacyclic form is triggered by some glycoprotein secreted by the intestin of the vector and recognized by the parasite during its stage in the lumen of the organ. Consequently, we studied the effect of some hexoses and its derivates on the metacyclogenesis of Trypanosoma cruzi, using a model of differentiation previously described by Isola et al. (6).

## MATERIALS AND METHODS

### Effect of sugars on morphogenesis

The differentiation model described by Isola et al. was used (6). Breafly, epimastigotes (4 x $10^6$ / ml) from biphasic medium (BP) were stimulated in Grace medium (GM) plus intestinal homogenate (IH) for 15 min at 28°C (stimulated epimastigotes), then transferred to GM for 15 days at 28°C. A batch of non stimulated parasites was transferred to GM as a control.

Morphogenesis was evaluated with the light microscope according to parasite motility, shape, and relative kinetoplast-nucleus position by counting at least 200 cells in wet preparations (6). It was expressed as the percentage of metacyclic forms, and it was estimated by quintuplicate every 48 hours.

Epimastigotes from biphasic medium were washed with 10 mM phosphate buffer (pH 7.2) containing 0.15 M NaCl. The pellet was suspended in 10 mM Tris-HCl buffer (pH 7.2) containing 0.25 M sucrose. A volume of 150 µl of hexose in Tris-HCl buffer was added to 1.5 ml of parasite suspension ($3x10^7$ cells). The final concentration of each sugar was 25 mM. The samples were incubated at 28°C for 20 min, and centrifuged at 3,000 rpm for 10 min. Each pellet was resuspended in GM + IH or in GM + IH plus each sugar at a concentration of 25 mM and incubated at 28°C for 15 min. After this stimulation in the presence of IH, the samples were centrifuged and transferred to 0.5 ml of GM, being incubated at 28°C for 10 days. The percentage of metacyclic forms was then evaluated as explained above.

The effect of high ionic strength on morphogenesis was tested in cultures containing 0.4 M NaCl.

In order to detect if a sugar stimulates the metacyclogenesis in the absence of IH, the epimastigotes were transferred to GM with each sugar, cultured at 28°C for 10 days, and morphogenesis was measured as explained above.

Chemical and enzymatic treatment of the parasites

Epimastigotes from biphasic cultures were washed with PBS and treated with agents that may modify cell membrane composition. For this purpose, $10^8$ cells were incubated in 1 ml of the buffer containing 0.08 units of neuraminidase (Sigma) at 37°C for 20 min. (7). After the incubation, the sample was diluted to 2.0 ml with cold PBS, and centrifuged for 10 min at 3,000 rpm. The cells were washed once with the buffer, and processed for the evaluation of morphogenesis.

The effect of sodium periodate was studied by incubating $10^8$ cells with the salt in a concentration 1 mM at 4°C for 15 min in the darkness. The reaction was stopped by doubling the volume of the sample with 2% glycerol in PBS buffer (8). The samples were centrifuged, washed once with PBS, and the parasites were processed for the evaluation of the morphogenesis with suitable controls.

Chemical and enzymatic treatment of the intestinal homogenate

The IH was prepared from the intestin of Triatoma infestans according to Isola et al. (5). A volume o 1 ml of filtrated IH (10 mg of proteins in PBS) was incubated with 10 mM sodium metaperiodate at 4°C for 6 h in the darkness. The reaction was stopped with the addition of glycerol to a final concentration of 0.2 M (9). The mixture was then dialysed against one liter of PBS at 4°C for 24 h, then diluted 1:9 (v/v) with the GM. The morphogenesis of the dialysed sample was evaluated together with suitable controls containing untreated IH.

To study the effect of trypsin, 150 ug of enzyme (Sigma) were added to one ml of IH (10 mg of protein), and the mixture was incubated at 37°C for 20 min. After the incubation, 150 ug of trypsin soybean inhibitor (Sigma) were added, and the morphogenesis was tried out as explained elsewhere.

The treatment with alkaline phosphatase was carried out according to Ullrich et al. (10). A volume of 1 ml of IH (10 mg of protein) was dialysed overnight against 50 mM Tris-ClH buffer (pH 7.5) containing 1 mM MgCl2. Alkaline phosphatase (Sigma type III) was added to a final concentration of 2.3 units per ml. The mixture was dialysed for 4 hours at 4°C, and for 6 hours at 37°C against the Tris-ClH buffer. The morphogenesis was then assessed together with controls containing alkaline phosphatase.

RESULTS

Table 1 shows the effect of sugars and its derivates on differentiation. In the GM supplemented with IH (GM + IH) 66% of parasites differentiated to the metacyclic form, while only 10% differentiated in the absence of IH. The presence of mannose in the medium inhibited metacyclogenesis to a level equal to that of controls without IH (column B).

The metacyclogenesis presented a moderate increase when the parasites were pre-washed with this sugar, and cultured in a sugar free medium (column A). Regarding the other sugars tested, only N-acetylgalactosamine and galactose 6-phosphate caused a moderate inhibition.

Table1: Effect of sugars, derivates and high ionic strength (0.4 M NaCl) on the Trypanosoma cruzi metacyclogenesis

| Compound | Percentage of metacyclics | |
|---|---|---|
| | A | B |
| Sugars (25 mM): | | |
| Sucrose | 66 ± 16 | 66 ± 7 |
| Mannose | 73 ± 12 | 10 ± 6 |
| Galactose | 55 ± 15 | 59 ± 6 |
| Fucose | 64 ± 14 | 58 ± 6 |
| N-Acetylglucosamine | 55 ± 15 | 60 ± 11 |
| N-acetylgalactosamine | 56 ± 17 | 45 ± 11 |
| Mannose 6-P | 56 ± 6 | 58 ± 7 |
| Galactose 6-P | 60 ± 8 | 47 ± 10 |
| Fructose 1,6-P | 65 ± 10 | 68 ± 8 |
| Salt (0.4 NaCl) | 15 ± 3 | |
| Controls: | | |
| Not stimulated with IH | 10 ± 3 | |
| Stimulated with IH | 66 ± 10 | |

Epimastigotes were washed with the sugar before stimulation in Grace medium supplemented with the intestin homogenate (IH) of Triatoma infestans. They were then cultured in the Grace medium without (A) or in the presence of the sugar (B). In the experiments with 0.4 M NaCl, stimulated epimastigotes were used. The data represent mean and standard deviation of three experiments run in quintuplicate (sugars) or of one experiment run in quintipulicate (sugar derivates and salt).

The addition of 0.4 M NaCl to cultures of stimulated epimastigotes strongly inhibited metacyclogenesis (15 ± 3%) while neither the salt nor the sugars had any effect on the differentiation of non stimulated epimastigotes. When the epimastigotes were treated with metaperiodate, the percentage of metacyclic forms decreased from 66 ± 10% for controls in GM + IH to 24 ± 1%. Neuraminidase had no effect (66 ± 10%). Treatment of IH with metaperiodate caused a decrease in the percentage of metacyclic forms from 71 ± 10% (IH stimulated controls) to 5 ± 1%. Similar results were obtained with trypsin treatment (5 ± 3%), while alkaline phosphatase, did not show a significative effect (58 ± 17%).

## DISCUSSION

The results show that only mannose causes a significative inhibition of metacyclogenesis. Treatment of IH with sodium periodate or trypsin inhibited stimulation of metacyclogenesis, suggesting that the IH contains one (or more) glycoprotein involved in the stimulation of epimastigote differentiation. In our opinion, the glycoprotein contains mannose, the ligand recognized by an affinity site located on the surface of the parasite. According to the results obtained with neuraminidase, sialic acid would not be indispensable for recognition of the differentiation factor.

In a previous report, Isola et al. (2) showed that stimulation of metacyclogenesis does not occur if the parasites are washed, and the IH removed from the medium during the first four hours of exposure. After this period, stimulation becomes irreversible indicating that the stimulating factor does not act immediately after exposure, and suggests us that it may be incorporated into the parasite by a specific pinocytic process.

It is interesting to observe that high ionic strength also inhibited metacyclogenesis, as it may be expected if the binding of the factor to plasmalemma occurs before the endocytic process.

Though not statistically significative, there was an increase of metacyclic forms with parasites washed with mannose. This could be interpreted as the result of an elimination from the surface of the epimastigote of compounds competing for the site of binding of the factor.

We believe our experiments may be useful to open new strategies to understand the metacyclogenesis, the acquirement of virulence, and mechanisms of host-parasite relationship.

Acknowledgments: This work was supported by a grant from the Government of the Provincia de Mendoza, Argentina, and from the Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET) of Argentina. We thank Mrs Nilda Pochettino for correcting the manuscript.

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