

## ROLE OF *TRYPANOSOMA CRUZI* LIPOPOLYSACCHARIDE ON HUMAN GRANULOCYTE BIOLOGICAL ACTIVITIES

LUIZ COSME COTTA MALAQUIAS; STANISLAWA SOFIA GOLDBERG\*;  
ANIBAL ANTONIO SILVA-PEREIRA & JOSE AUGUSTO NOGUEIRA-MACHADO

Departamento de Bioquímica-Imunologia \*Departamento de Microbiologia Instituto de Ciências Biológicas,  
Universidade Federal de Minas Gerais, Caixa Postal 2486, 31270 Belo Horizonte, MG, Brasil

It is well known that bacterial lipopolysaccharide (LPS) can influence the oxidative metabolism, as well as, the phagocytic function of human polymorphonuclear cells (PMN) (R. A. Proctor., 1979, *Infect. Immun.*, 25: 912-921; A. Kapp et al., 1987, *Infect. Immun.*, 41: 294-301). The oxidative changes that occur in LPS-challenged PMN are followed by the generation of reactive derived radicals that are related to the PMN bactericidal activity (B. M. Babior, 1978, *N. Engl. J. Med.*, 298: 659-668). Neutrophils from patients with chronic Chagas' disease, but not from normal subjects, exhibited a significant decrease in chemotaxis and nitroblue tetrazolium (NBT) reduction (J. C. Voltarelli et al. 1990, *Rev. Inst. Med. Trop. São Paulo*, 32: 240-248). We have previously described and characterized LPS from *T. cruzi* Y and CL strains (S. S. Goldberg et al., 1983, *Int. J. Parasitol.*, 13: 11-18). In the present study, the effect of *T. cruzi* LPS (Y strain) and *Escherichia coli* 055:B5 LPS on tetrazolium salt MTT dye reduction and generation of chemiluminescence by normal human granulocytes were evaluated.

We used the Y strain of *T. cruzi* and a single lot of *T. cruzi* LPS was obtained as previously described (S. S. Goldberg et al., *loc. cit.*). The human granulocytes were purified in a ficoll-hypaque gradient (H. M. S. Bicalho et al., 1981, *J. Immuno. Meth.*, 40: 115-116). The opsonization of zymosan particules was achieved by incubation of the membrane-active substance and fresh human serum in Hanks' solution at 37 °C for 30 min. The quantitative MTT dye reduction was performed as follows: human PMN ( $5 \times 10^5/100 \mu\text{l}$ ) and 5  $\mu\text{g}$  of LPS either from *T. cruzi* or from *E. coli* were incubated

for 5 min at 37 °C. Following incubation, 20  $\mu\text{l}$  of MTT (5 mg/ml in PBS) and 25  $\mu\text{l}$  of opsonized zymosan (1.3 mg/ml) were added and the mixture were incubated for 120 min at 37 °C. The control in absence of opsonized particules was performed simultaneously. The reaction was stopped by adding 1.5 ml of isopropanol/HCL (0.04 N) and the absorbance was read at 570 nm. The chemiluminescence assay was performed by incubation of PMN ( $5 \times 10^5/100 \mu\text{l}$  in Hanks') with LPS (5  $\mu\text{g}$ ) for 30 min at 37 °C. Opsonized zymosan (50  $\mu\text{l}$ ) and 500  $\mu\text{l}$  of 0.4 mM luminol solution was added into unsealed polystyrene luminescence tubes and chemiluminescence measurements were performed in a luminometer 1250-101 (LKB-Produkter AB, 5-16125 Bromma, Sweden).

The data from Table show that opsonized zymosan-induced MTT reduction by human granulocytes was significantly increased when compared with resting cells ( $p < 0.05$ ). The *E. coli* LPS and *T. cruzi* LPS were not able to potentiate or to inhibit the MTT reduction in these cells. The Fig. show the time course of chemiluminescence response by phagocytic cells (PMN). This response in the absence of LPS begins about 3 min after addition of opsonized zymosan and reaches maximum values at 12 min. However, the incubation of PMN with either *T. cruzi* LPS or *E. coli* LPS before stimulation with opsonized particules resulted in a consistent and statistically significant reduction of their ability to generate chemiluminescence ( $p < 0.05$ ) after 6 min of reaction. The kinetics for *T. cruzi* LPS and *E. coli* 055:B5 LPS are strikingly similar ( $p > 0.05$ ).

Our results demonstrate for the first time the effect of LPS from *T. cruzi* (Y strain) on human granulocyte function. The effects with *T. cruzi* LPS were similar to those presented by *E. coli* 0:55:B5. The MTT dye reduction by PMN was not affected by incubation with

This research was supported by grants from CNPq, FAPEMIG PRPq-UFMG and FINEP.

Received 17 November 1990.

Accepted 18 July 1991.

TABLE

Effect of *Trypanosoma cruzi* and *Escherichia coli* 055:B5 LPS on MTT dye reduction

Experiments	Absorbance 570nm $\pm$ S.E.
G + Hanks' (Resting cells)	0.219 $\pm$ 0.19
G + <i>T. cruzi</i> LPS	0.199 $\pm$ 0.27 <sup>a</sup>
G + <i>E. coli</i> LPS	0.235 $\pm$ 0.39 <sup>b</sup>
G + opsonized zymozan (OZ)	0.434 $\pm$ 0.04 <sup>c</sup>
G + <i>T. cruzi</i> LPS + OZ	0.365 $\pm$ 0.29 <sup>d</sup>
G + <i>E. coli</i> LPS + OZ	0.468 $\pm$ 0.08 <sup>e</sup>

G = granulocytes from normal subjects.

<sup>a</sup> P and <sup>b</sup> P: were not significant when compared with control.

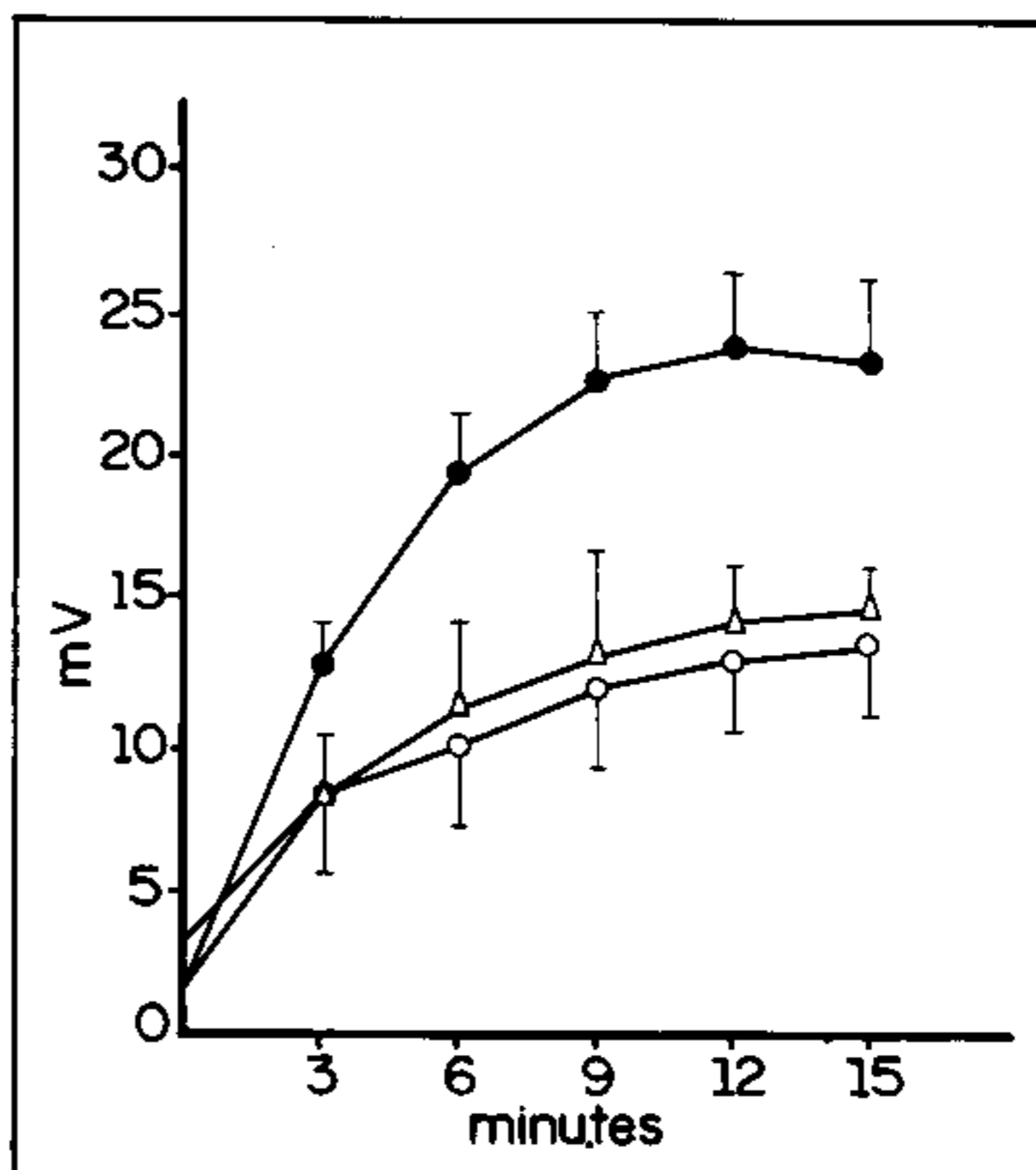
<sup>c</sup> P < 0.05: compared with control.

<sup>d</sup> P and <sup>e</sup> P: were not significant when compared with zymozan stimulated cells (c).

<sup>a</sup> P and <sup>d</sup> P: were not significant when compared with b and c LPS-induced groups, respectively.

LPS either from *T. cruzi* or from *E. coli*. Both kind of LPS, however, were able to induce a highly significant decreasing in generation of chemiluminescence. We do not know at the moment the reason for the significant reduction of chemiluminescence by opsonized zymozan phagocytosing-granulocytes in the presence of *T. cruzi* LPS. The mechanism of immunosuppression in trypanosomiasis is poorly understood and we have hypothesized that this LPS-induced decreasing in granulocyte function could be associated to the *in vivo* scape mech-

anism of *T. cruzi*. However, further experiments are needed to demonstrate the validity of this hypothesis.



Effect of *Trypanosoma cruzi* and *Escherichia coli* 055:B5 LPS on human granulocyte chemiluminescence — ●: control — (PMN + opsonized zymozan); Δ: experiment — (PMN + *T. cruzi* LPS + opsonized zymozan); ○: experiment — (PMN + *E. coli* LPS + opsonized zymozan). The results above represent the average of six experiments  $\pm$  S. E. After 6 min reaction, the values obtained in the presence of LPS were significant ( $p < 0.05$ ) when compared to the control (Student "t" test).