

## A SIMPLE METHOD FOR ASSESSING THE BINDING OF CONCAVALIN A TO MONONUCLEAR CELL SURFACES: NO INTERFERENCE OF VISCERAL LEISHMANIASIS SERUM ON THIS BINDING

M. BARRAL-NETTO & A. BARRAL\*

*We report a simple method for evaluating the binding of concanavalin A (ConA) to human peripheral blood mononuclear cells (PBMC). The binding is evidenced by an immunoenzymic assay using peroxidase-conjugated immunoglobulins of a rabbit anti-ConA serum. Using the method we show that sera from patients with American leishmaniasis do not interfere with binding of ConA to PBMC.*

Key words: concanavalin A – mononuclear cells – visceral leishmaniasis

The presence of serum suppressive factors capable of inhibiting lymphocyte clonal expansion *in vitro* has been reported in parasitic diseases (Colley et al., 1977; Mota-Santos et al., 1977; Ottesen & Pointdexter, 1980; Rocklin et al., 1980; Barral-Netto et al., 1982; Wyler, 1982; Carvalho & Bacelar, 1983; Barral et al., 1986) as well as in other conditions (Winfield et al., 1976; Mehra et al., 1979; Tsuyugushi et al., 1980). The possibility of sequestration of the antigen by antibodies in the serum must always be considered when studying antigen-driven lymphocyte proliferation. Such hypothesis is less likely when the phenomenon deals with stimulation by mitogens. Eventhough, anti-mitogen antibodies may appear due to polyclonal B cell activation. The binding of the mitogen to the cellular surface must be demonstrated in some situations. We report herein a method for evaluating the binding of concanavalin A (ConA) to peripheral blood mononuclear cells (PBMC).

PBMC were obtained from normal volunteers' heparinized venous blood layered onto a Hypaque-Ficoll gradient (LSM; Litton Bionetics, Kensington, MD, USA). After washing, PBMC were resuspended in RPMI 1640 culture medium (GIBCO; Grand Island, NY, USA), with 200IU/ml of penicilin and 100µg/ml of streptomycin. Cells were then incubated in volumes of 1ml ( $10^6$  cells) at different concentrations of ConA (Sigma, St. Louis, MO, USA) for 1 hour, at 37°C in a humid atmosphere with 5% CO<sub>2</sub>. Cells were washed three times with RPMI-1640. In some experiments cells were washed twice with a solution of 20mg/ml of alpha-methyl mannopiranoside (alpha-MM; Calbiochem-Behring Corp. La Jolla, CA, USA), to remove ConA before washing with RPMI 1640. Incubation with peroxidase-conjugated immunoglobulins of a rabbit anti-concanavalin A serum (Dako Corporation, Santa Barbara, CA, USA), was performed at an 1:100 dilution in medium containing 10% autologous plasma and left to proceed for 30 minutes at 37°C. Cells were then transferred to round-bottom microtiter plates (0.1ml/well; eight replicates per tube) and washed twice. The last resuspension was made with 200µl/well of ELISA substrate solution: 0.04% o-phenylenediamine (Sigma; St. Louis, MO, USA), and 0.012% hydrogen peroxide in phosphate-citrate buffer, pH 5.0. After incubation at room temperature, for 30 min, in the dark, the optical density (492nm) was determined in an automatic reader (Titertek Multiskan; Flow Laboratories McLean, VA, USA).

Controls consisted of tubes without ConA or incubated with ConA but without peroxidase conjugate. The use of 10% autologous plasma or fetal calf serum during incubation with peroxidase-conjugated anti-ConA immunoglobulin reduced background optical densities, probably due to a decrease in the non-specific reaction of the conjugate with Fc receptors on cell surfaces. The use of peroxidase-conjugated Ig gave similar results both at 1:100 to 1:200 dilutions. Cells ( $5 \times 10^4$  cells/well) incubated with 10µg of ConA/ml and washed with RPMI 1640 produced an OD<sub>492nm</sub><sup>1cm</sup> of 0.610; when ConA-incubated cells were washed twice with alpha-MM (20mg/ml) the OD decreased to 0.393, a value which was similar to those obtained with cells without ConA (OD of 0.361).

Dose-response curves were obtained with  $1.5 \times 10^3$  or  $5 \times 10^4$  cells/well (Table I); in both groups it was possible to evaluate the higher concentrations of ConA but only at the lower cell density it was possible to detect the lower ConA concentrations.

This work received support from CNPq and from United States Public Health Service Grant AI-16282.

Centro de Pesquisas Gonçalo Moniz – FIOCRUZ/Universidade Federal da Bahia and \*Laboratório de Imunologia, Hospital Prof. Edgard Santos, UFBA – Rua Valdemar Falcão 121, Brotas, 41930 Salvador, BA, Brasil.

Received for publication March 10th and accepted July 3rd, 1986.

TABLE I

Optical densities (492 nm) from peripheral blood mononuclear cells incubated with different concentrations of Concanavalin A

ConA ( $\mu\text{g/ml}$ )	OD 492 nm <sup>(a)</sup> ( $\bar{X} \pm \text{S.E.M.}$ )			
	1.5 x 10 <sup>4</sup> cells/ml		5 x 10 <sup>5</sup> cells/ml	
1	0.015	0.009	— (b)	
5	0.019	0.005	—	
10	0.034	0.002	0.192	0.022
50	0.096	0.005	0.582	0.058
100	0.097	0.004	0.742	0.030

(a) OD of cells incubated with ConA minus OD of cells in mitogen free medium. Eight replicates in each measurement.

(b) — = Measurement not different from that obtained with cells without ConA.

The test described here was able to detect the binding of ConA to PBMC at the concentrations used in lymphocyte proliferative assays (5 to 20  $\mu\text{g/ml}$  as optimal dose). This circumstance renders it suitable for evaluation of such assays.

We have tested the possible effect of sera from American visceral leishmaniasis (AVL) patients, which were able to decrease ConA-driven *in vitro* lymphocyte proliferation, on the binding of ConA to PBMC surface. Cells from a normal volunteer (10<sup>6</sup> cells/ml) were incubated with 50  $\mu\text{g/ml}$  of ConA in the presence of 10% sera from AVL patients (n = 6) or normal human sera (three heterologous samples besides the autologous serum). OD<sub>492 nm</sub> obtained with NHS was  $1.36 \pm 0.43$  ( $\bar{X} \pm \text{S.E.M.}$ ), similar to the value obtained with AVL sera ( $1.31 \pm 0.25$ ).

Our results show that sera from AVL patients do not interfere with the binding of ConA to PBMC. The lower proliferative response of lymphocytes to ConA in the presence of AVL sera, as compared to normal human sera, probably depends on interference with subsequent(s) step(s) of cell activation.

## RESUMO

Descrevemos um método simples para determinação da ligação da Concanavalina A na superfície de células mononucleares do sangue periférico humano. A ligação é revelada por método imuno-enzimático utilizando imunoglobulina, conjugada à peroxidase, obtida de soro de coelho anti-ConA.

## REFERENCES

- BARRAL, A.; CARVALHO, E.M.; BADARÓ, R. & BARRAL-NETTO, M., 1986. Suppression of lymphocyte proliferative responses by sera from patients with American visceral leishmaniasis. *Am. J. Trop. Med. Hyg.*, 35 :735-742.
- BARRAL-NETTO, M.; HOFSTETTER, M.; GUSTAVO DOS SANTOS, J.; CHEEVER, A.W. & OTTESEN, E.A., 1982. *Schistosoma mekongi* infection in man: cellular immune responses and modulating mechanisms. *Clin. Exp. Immunol.*, 47 :65-73.
- CARVALHO, E.M. & BACELLAR, O.A., 1983. Lymphocyte reactivity to mitogens in American visceral leishmaniasis. *Braz. J. Med. Biol. Res.*, 16 :35-41.
- COLLEY, D.G.; COOK, J.A.; FREEMAN, G.L.JR.; BARTHOLOMEW, R.K. & JORDAN, P., 1977. Immune responses during human schistosomiasis mansoni. III. Regulatory effect of patient sera on human lymphocyte blastogenic responses to schistosome antigen preparations. *Am. J. Trop. Med. Hyg.*, 26 :917-925.
- MEHRA, V.; MANSON, L.H.; FIELDS, S.P. & BLOOM, B.R., 1979. Lepromin-induced suppressor cells in patients with leprosy. *J. Immunol.*, 123 :1813-1817.
- MOTA-SANTOS, T.A.; TAVARES, C.A.P.; GAZZINELLI, G. & PELLEGRINO, J., 1977. Immunosuppression mediated by adult worms in chronic schistosomiasis mansoni. *Am. J. Trop. Med. Hyg.*, 26 :727-732.
- OTTESEN, E.A. & POINTDEXTER, R.W., 1980. Modulation of the host response in human schistosomiasis. II. Humoral factors with inhibit lymphocyte proliferative responses to parasite antigens. *Am. J. Trop. Med. Hyg.*, 29 :592-597.
- ROCKLIN, R.E.; BROWN, A.P.; WARREN, K.S.; PELLE, R.P.; HOUBA, V.; SIONGOK, T.K.A.; OUMA, J.; STURROCK, R.F. & BUTTERWORTH, A.E., 1980. Factors that modify the cellular-immune response in patients infected by *Schistosoma mansoni*. *J. Immunol.*, 125 :1916-1923.

- TSUYUGUSHI, I.; SHIRATSUCHI, H.; TERAOKA, O. & HIRANO, T., 1980. Increase in T cells bearing IgG Fc receptor in peripheral blood of patients with tuberculosis by "in vitro" stimulation with purified protein derivative. *Am. Rev. Resp. Dis.*, 121 :951-957.
- WINFIELD, J.B.; WINCHESTER, R.J.; WERNER, P. & KUNKEL, H.G., 1976. Antibodies to lymphocyte and the estimation of B and T cells in the connective tissue disorders. In: *The Role of Immunological Factors in Infectious, Allergic and Autoimmune Process* (ed. by R.F. Beers, Jr. and E.G. Bassett). Vol. 1, Chap. 13. Raven Press. N.Y.
- WYLER, D.J., 1982. Circulating factor from a Kala-Azar patient suppresses "in vitro" anti-leishmanial T cell proliferation. *Trans. R. Soc. Trop. Med. Hyg.*, 76 :304-306.