Immunology of onchocerciasis. II. Modulatory effects of serum and cell factors on lymphocyte proliferative responses to mitogens and alloantigens

G.E. Pérez-Rojas*, M. Valderrama*, C. Echeverría*, I. Machado*, C.E. Contreras*, L. Yarzábal and N.E. Bianco***

* Clinical Immunology National Center (SAS/UCV). The Pathology Institute and the WHO Collaborative Center in Clinical Immunology (CECOIC). WHO/PAHO-SAS.
** Centro Amazónico para Investigación y Control de Enfermedades Tropicales "Simón Bolívar" (CAICET). Apartado 593, Puerto Ayacucho, Territorio Federal Amazonas, Venezuela.

Inmunología de la oncocercosis. II. Efectos modulatorios del suero y factores celulares sobre la respuesta proliferativa de los linfocitos frente a mitógenos y aloantígenos

En 19 pacientes infectados por *Oncocerca volvulus* (OV), todos ellos indios Yanomami residentes en un foco endémico de la selva amazónica venezolana, se investigó la capacidad proliferativa de las células mononucleadas de sangre periférica (previamente precultivadas) frente a PHA y aloantígenos. En general, la respuesta proliferativa se hallaba conservada salvo en 8 de 18 pacientes, que mostraban valores bajos frente a aloantígenos. Un hallazgo significativo fue la acción bimodal (inhibición o potenciación) ejercida por el suero autólogo sobre la reactividad de las células. El suero autólogo causaba inhibición de la respuesta proliferativa frente a aloantígenos y frente a PHA en el 66 % y 26 % de los casos, respectivamente; el efecto potenciador se observó en el 21 % de las respuestas frente a PHA. Por experimentos de cocultivos se halló que también las células de los pacientes ejercían una influencia bimodal (inhibición o potenciación) sobre la respuesta proliferativa de células mononucleadas normales frente a aloantígenos. Los factores séricos o celulares causantes de estos efectos pueden deberse a la presencia de antígenos o anticuerpos libres, a complejos inmunes circulantes o a citocinas liberadas *in vitro* por células preestimuladas *in vivo*.

We explored the reactivity of precultured cells stimulated with PHA and alloantigens in Yanomami indians infected with *O. volvulus*, from an endemic focus in the Venezuelan Amazonian forest. The capacity to proliferate was preserved in most cases with the exception of 8/18 which exhibited low proliferative response to alloantigens in the mixed lymphocyte culture (MLC).

A significant finding was the bimodal action (inhibition or enhancement) over cell reactivity exherted by autologous serum factors. This influence resulted in the inhibition of the response to alloantigens in 66 % and to mitogen in 26.3 % of the patients; enhancement was noted in 21 % of PHA responses.

Remarkable was to find that co-culturing of patients cells whithin a normal control MLC, also showed a bimodal action, enhancing or inhibiting the proliferative response to alloantigens.

The probably nature of the observed serum and/or cell immunomodulatory factors may be related to free antigens or antibodies, circulating immune complexes or else to cytokines released *in vitro* by an already *in vivo* primed cells.

INTRODUCTION

The role of cell mediated immunity (CMI) in parasitic diseases has been recently stressed[1-3]. However, little is known on the CMI effector and immunoregulatory mechanisms in onchocerciasis. Cell reactivity has been tested *in vivo* and *in vitro*[4-5] and recently Greene et al[6] suggested the of non-specific supression of antigen induced lymphocyte blastogenesis in *O. volvulus* infection. In our group, free circulating antigen has been associated with depression of delayed type skin test to *O. volvulus*[7].

These observations prompted us to investigate CMI in Yanomami Amerindians infected with *O. volvulus*, exploring simultaneously different effector phases of CMI response and the possible influence of serum and/or cell factors in the *in vitro* cell reactivity.

PATIENTS AND METHODS

Selection of population studied

Nineteen untreated Yanomami inhabitants of the Venezuelan slopes of the Sierra Parima affected with onchocerciasis were selected; control group included Latinoamerican Caucasoid and mestizo normal volunteers from Caracas, since Yanomami individuals free of filariasis have not been identified by our group.

Reagent standarization

Lymphocyte preparations, cell preculture conditions and blastogenec assays have been previously described[8]. Briefly, peripheral blood mononuclear leukocytes (PBML) were obtained by Ficoll-Hypaque density-gradient centrifugation of fresh heparinized blood[9], washed two times and adjusted to desired concentration in RPMI 1640 medium (Microbiologi-

Correspondencia: Gloria E. Pérez-Rojas, Inmunología Clínica, Apartado 50647, Correos de Sabana Grande, Caracas, Venezuela.

TABLE I **Effect of autologous serum (AS) on the proliferative response to PHA**

Patients	n	NHS $\bar{X} \pm SE$	p	AS $\bar{X} \pm SE$	I or E Range
Total	19	23.934 ± 2.501	NS	22.302 ± 2.824	-
Inhibition	5	26.900 ± 4.252	< 0.025	12.685 ± 2.858	23 %-78 %
Enhancement >20 %	4	20.933 ± 6.935	< 0.025	34.655 ± 7.743	30 %-248 %
No modification	10	23.652 ± 3.530	NS	22.169 ± 2.932	9 %-16 %
Controls	6	20.802 ± 2.425	NS	17.877 ± 2.696	7 %-20 %

Responding cells: 5×10^4 per well. PHA: Optimal dose. I or E: Inhibition or Enhancement by AS when compared to NHS, expressed in %. p: p value (Student's T test). NHS: Normal human serum. AS: Autologous serum. NS: non significant.

cal Associated, Bethesda, Maryland) supplemented with 25 mM Hepes buffer, 2 mM glutamine, 100 u/ml penicillin, 100 ug/ml streptomycin and 10 % heat inactivated pooled male serum (NHS) or autologous serum (AS).

Cultures were carried out in round bottomed microtiter plates (Falcon Plastics, Inc., Oxnard, California), each well containing a final volume of 0.2 ml. All cultures were maintained at 37° C in a humidified atmosphere of 5 % CO_2, and were pulsed with 1 uCi tritiated thymidine, 18 hours prior to harvesting with a Mash II apparatus (Microbiological Associated, Maryland).

In all cases, PBML (1×10^6 cells/ml) from patients and controls were precultured in RPMI medium supplemented as above but with 2 % NHS, for 18 hours at 37° C. Preculture fluids were collected and stored for analysis; PBML were counted and adjusted to desired concentration in fresh medium.

In vitro assays

Lymphocyte transformation test (LTT) was studied by 5×10^4 PBML stimulated with a previously determined optimal dose of PHA (PHA-M, Calbiochem); cultures were pulsed as above and harvested on day 3 (72 hours).

PBML response to alloantigens was examined in one-way mixed lymphocyte culture (MLC): 5×10^4 responding and 5×10^4 mitomycin-treated pooled cells from 3 normal individuals, were cultured for 6 days when pulsed and harvested as stated above.

To search for possible suppressor cells in patients with onchocerciasis, 5×10^4 cells from these individuals were cocultured with 5×10^4 responding and stimulators obtained from controls. Results were compared with the same experiment where patient cells were substituted by 5×10^4 third party control.

Immune complexes (IC) in preculture fluid

IC were searched by the solid phase Clq microassay as previously standarized in our laboratory[10].

Lymphocyte proliferative response

It was analyzed and expressed in cpm when a single experiment was shown. Relative proliferation index (RPI), as described by Dean et al[11], was used when different experiments were compared. RPI is defined as the ratio between the net cpm of the patient and the mean net cpm of three of more normal subjects assayed simultaneously. Cut off values to define depressed response were established as the bottom ten percentile of normal RPI values for 150 controls, as previously reported in our laboratory by Blanca et al[12]. This procedure allowed us to define depressed or enhanced proliferative responses with accuracy.

The results of inhibition or enhancement, when expressed in percentage, were obtained in the following manner:

$$1 - \frac{\text{cpm of culture in the presence of patient cell}}{\text{cpm of culture in the presence of control cell}} \times 100$$

Statistical analysis

T-statistical evaluation for paired observations and two sample test, and Student's distribution program were performed using a Hewlett-Packard H-P programmable calculator.

TABLE II **Proliferative response to alloantigens in MLC**

Patients	NHS	AS	(% I)
1	0.72	0.76	
2	2.24	1.26	(44)
3	1.19	1.14	
4	1.01	0.79	(22)
5	0.87	0.76	(13)
6	1.10	0.95	(14)
7	0.90	0.65	(28)
8	1.34	0.94	(30)
9	1.02	0.57	(44)
10	1.25	0.39	(69)
11*	0.39	0.36	
12*	0.54	0.30	(45)
13*	0.61	0.07	(89)
14*	0.28	0.30	
15*	0.32	0.13	(59)
16*	0.61	0.39	(36)
17*	0.50	0.36	(28)
18*	0.52	0.30	(58)
$\bar{X}$	0.86	0.58	(33)

$\bar{X}$ RPI of 5 controls in NHS: 0.99.
* RPI $\leq$ 0.66 in NHS.
(% I) Percent inhibition by AS over NHS.
RPI: Relative proliferation index (see patients and methods and Ref 11 and 12).
NHS: Normal human serum. AS: Autologous serum.
MLC: mixed lymphocyte culture.

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