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T-Lymphocyte Behaviour Modification *in vitro* by Cancer Patient Sera

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Cell mediated immune response blocking factor(s) in the serum of colon adenocarcinoma patients inhibit blast transformation of lymphocytes from normal individuals. The blocking capacity of the sera has been shown to correlate with the infiltration of the tumor. This correlation suggests that these phenomena may be mediated by identical serum factors and through a common cell receptor present in the lymphocytes of normal individuals.

Key words: Blast transformation, Cancer, Serum.

It is well known that sera from some cancer patients have the ability to inhibit the cell mediated immune response against tumor cells (7, 8).

The presence of inhibitory factor(s) has been demonstrated in the serum of many patients with malignant tumors (2, 4, 6, 14) and can be detected by several techniques (9-12). However, the physiological role of this factor(s) has not been established yet.

In this paper the results of experiments performed in order to elucidate whether or not sera from patients bearing colon adenocarcinoma can block blast transformation of normal lymphocytes from healthy donors, in response to phytohaemaglutinin, are shown.

Materials and Methods

Sera. A collection of sera from 25 individuals bearing colon adenocarcinoma was used. As control, AB Rh^+ serum was used. Both, patients and control sera were heat-inactivated at 56°C during 30 min to remove the complement's activity, filtered through a 0.22 μ pore

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size millipore filter (Millipore, USA) and stored at — 20°C until used. Patient's sera were grouped into B and C stages according to the infiltration of the tumor (5).

Culture medium. Culture medium RPMI 1640 (Flow, England) was used. It was supplemented with 2 g/100 ml of 200 mM L-glutamine (Flow), 250 μg/ml of amoxiciline, 4.4 % sodium bicarbonate (Wellcome, England) and 10 % heat inactivated foetal calf serum (Flow).

Isolation of lymphocytes. Lymphocytes were obtained from peripheral blood of 5 healthy donors. To isolate from the technique by BOYUM (3). Briefly, 20 ml of blood of each donor were collected and diluted 1/1 (v/v) with phosphate buffered saline (PBS) (Oxoid, England) and defibrinated. Afterwards the lymphocytes were isolated by centrifugation on a density gradient of Urograf-Ficoll (Pharmacia, Sweden) (d=1.077 g/l), washed 3 times in PBS and adjusted to 10^6 cells/ml in culture medium.

Lymphocytes culture. Two hundred μl/well were dispensed in a 96 wells microtiter plate (Linbro, USA). Then, 0.30 μg/well of phytohaemagglutinin (PHA) (Difco, USA) were added. Finally, 20 μl of each serum were added to the corresponding wells by triplicate (1).

Plates were incubated for 48 h at 37°C in an atmosphere containing 95 % air and 5 % CO_2. After this incubation, cells were labelled with 1 μCi/well of tritiated thymidine (1 μCi = 37 kBq) (Amersham) and incubated in the same conditions during 16 h and collected by using a multiharvester (Flow). The radioactivity uptake was determined in a scintillation counter (LKB Rackbeta, Finland) following the technique described by PEÑA *et al.*, (15).

Results and Discussion

Table I shows the results obtained from group B patient sera. Three out of 13 serum samples are shown to significantly reduce the proliferative response of lymphocytes from normal individuals to PHA. By contrast, results obtained from group C patients show a very homogeneous pattern. Nine out of 12 sera are able to inhibit the normal lymphocyte response to PHA (table II).

A statistical analysis of these results suggests a correlation between the infiltration of patient's tumor and the ability of their sera to inhibit PHA stimulated proliferation of normal lymphocytes.

A Braun's T test analysis of the mean value of the response in group B (86.9 % of the control) and group C (77.4 % of the control) showed a significant difference ($p < 0.01$). The results compiled in tables I and II provide evidences for a very uniform response from 5 different donors' lymphocytes when stimu-

Table I. *Response to PHA from normal lymphocytes cultured in the presence of serum from stage B colon adenocarcinoma bearing Patients (%).*
Each value represents the mean of 3 experiments performed with lymphocytes of 5 different donors.

Patient number	$\bar{x} \pm$ S.D.	Significance
1	86.4 ± 14.1	N.S.
2	82.4 ± 21.4	N.S.
3	93.2 ± 14.2	N.S.
4	100.0 ± 8.3	N.S.
5	82.4 ± 19.5	N.S.
6	87.9 ± 7.9	N.S.
7	75.3 ± 14.1	N.S.
8	86.8 ± 6.4	*
9	71.1 ± 5.7	**
10	85.7 ± 3.7	**
11	85.5 ± 12.7	N.S.
12	92.7 ± 4.4	N.S.
13	100.5 ± 8.9	N.S.

* $p < 0.05$; ** $p < 0.01$; N.S.: Not significant.

Table II. *Response to PHA from normal lymphocytes cultured in the presence of serum from stage C colon adenocarcinoma bearing patients (%).*
Each value represents the mean of 3 experiments performed with lymphocytes of 5 different donors.

Patient number	$\bar{x} \pm$ S.D.	Significance
1	73.5 ± 9.3	*
2	71.5 ± 10.7	*
3	70.9 ± 9.7	*
4	75.7 ± 9.9	*
5	78.5 ± 10.2	*
6	73.1 ± 7.0	**
7	92.4 ± 11.5	N.S.
8	64.0 ± 17.8	*
9	86.3 ± 15.7	N.S.
10	85.4 ± 10.4	N.S.
11	76.9 ± 7.9	*
12	80.6 ± 5.4	**

* $p < 0.05$; ** $p < 0.01$; N.S.: Not significant.

lated in the presence of serum from the same patients. This observation suggests the inhibition may be mediated by identical serum factor(s) that may act through a cellular receptor common to all the lymphocytes tested.

These data support the findings of NISHIO *et al.* (13) on bladder carcinoma, who reported that the capacity of patient sera to reduce the blast transformation in response to PHA of normal lymphocytes correlates with the infiltration of the tumor.

Resumen

Se estudia en sueros de pacientes portadores de adenocarcinoma de colon la capacidad para inhibir la transformación linfoblástica de linfocitos de sangre periférica de sujetos sanos en respuesta a la fitohemaglutinina. Existe una estrecha correlación entre el estadio infiltrativo del tumor y la capacidad bloqueante de los sueros. Del patrón de respuestas observado puede inferirse que el receptor linfocítico, a través del cual pudiera mediarse la inhibición de la respuesta a la fitohemaglutinina, es único y está presente en los linfocitos de sujetos normales.

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