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A Comparative Study Between Fc Receptor Bearing Cells and Antibody Dependent Cell Cytotoxicity in Cancer Patients

F. Gutiérrez, E. Sánchez-Cantalejo, E. García-Olivares, F. Garrido and C. Osorio

Departamento de Fisiología
Sección de Inmunología
Facultad de Medicina
Granada (España)

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The capacity of 14 cancer patients lymphoid cells to destroy antibody-coated target cells (antibody-dependent cell cytotoxicity ADCC), and to form rosettes with immunoglobulin-coated erythrocytes (EA) was compared. The lymphocyte functions were investigated immediately prior to chemo-immunotherapy.

Our observations show a correlation between the percentage of EA-rosette forming cells and ADCC activity of cancer patients and controls. However, the regressive lines were different in both groups. Interestingly, cancer patients with a lower. EA rosette forming cells than controls, showed higher cytotoxic activity.

Minute amount of 7 S antibodies are able to kill target cells in the presence of a subpopulation of lymphoid cells, bearing Fc or C'3 receptors (2, 5, 8, 10, 12, 15). The cytotoxic reaction does not require complement. The biological significance of the cells responsible for the antibody-dependent cell cytotoxicity (ADCC) is poorly understood, despite data supporting the idea that it plays an important role in tumour rejection as well as in autoimmune diseases (7, 13, 16).

Comparative studies between the percentage of Fc receptors and ADCC have been done in rat lymphoid organs and no relationship has been reported (6). We present here the results obtained when a population of cancer patients were compared with healthy individuals in relation to the capacity for generating ADCC by Fc receptor bearing cells from peripheral blood.

Materials and Methods

Patient. Fourteen cancer patients aged between 25 and 65 (mean 48) have been studied. The patients had not been subjected to chemotherapy or radiotherapy before the test was performed. Twenty healthy donors aged between 16 and 60 were used as controls.

Preparation of effector cells from human peripheral blood. Lymphocytes were isolated from 10 ml of defibrinated human peripheral blood in a density gradient centrifugation (1). Cells were washed three times in Hanks solution and the concentration adjusted to the required experimental condition in Eagle's Medium, 2.5 % sodium bicarbonate (Wellcome) and 10 % inactivated faetal calf serum (Difco).

Target cells. Chicken erythrocytes were obtained from axillary veins. 20 μl of concentrated erythrocytes were labelled with $^{51}CrO_4Na_2$ (JEN, Sapain) for 1 hour at 37° C and washed twice in a large volume of Hanks solution. The final concentration of CRBC for the assay was 5×10^3/0.2 ml.

Heteroantisera (RACA). New Zealand rabbits received 10 intravenous injections of CRBC (10 % solution in PBS) in a period of 3 weeks at doses of 1 ml/kg weight. RACA (rabbit anti chicken antibody) was obtained two days after the last injections, heat inactivated and stored at —30° C.

EA rosetting technique. Chicken red blood cells (5 %) were incubated with CRBC heteroantibody at non agglutinating dilutions (1/750) for 30 min at 37° C, washed twice in Hanks solution and adjusted to a final concentration of 10 % (EA). A mixture of lymphocytes (1.5×10^6/0.25 ml) and 0.25 ml EA was spun down at 1500 rpm, for 5 min and incubated at 37° C for 15 min. Rosette forming cells were counted in phase contrast microscopy.

Antibody dependent cell cytotoxicity assay (ADCC). Round bottom polystirene tubes (11 × 55 mm) were used. The assay was performed in triplicate samples containing 5×10^3/0.2 ml ^{51}Cr labelled CRBC as target cells and 0.2 ml of inactivated RACA at various dilutions. The mixture was incubated at room temperature for 10 min and effector cells (0.2 ml) (PBL) added to get a final ratio effector/target of 100:1. The mixture (CRBC, RACA and PBL) was incubated 4 hours at 37° C in an atmosphere of 5 % CO_2.

0.5 ml of supernatant was obtained after centrifugation of the tubes at 1.000 rpm and used to count the radioactivity in an LKB gammas counter. Sheep red blood cells were added to the tubes in order to decrease the spontaneous ^{51}Cr release.

The percentage of citotoxicity was calculated according to the formula:

% ADCC = cpm supernatant with antibody — cpm supernatant without antibody /total cpm × 100

Results

Titration of RACA heteroantiserum. In order to select the optimal dilution of RACA in the ADCC system the rabbit anti-chicken antibody was titrated, keeping constant the ratio PBL/CRBC in 100 : 1. The highest cytotoxicity appeared at 5×10^3 (fig. 1). None of the dilutions used were cytotoxic in the absence of effector cells and there was no cytotoxicity with normal rabbit serum instead of RACA.

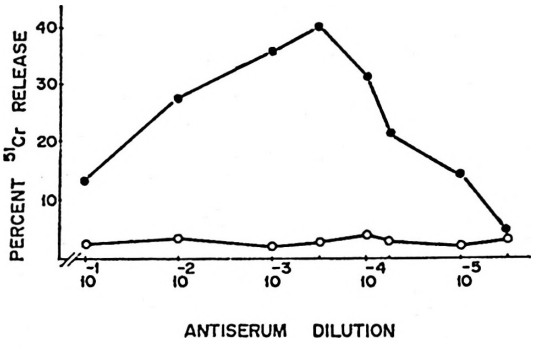

Fig. 1. *Titration of rabbit anti chicken antiserum (RACA) in ADCC (●—●) PBL: CRBC ratio 100/1.*
Non immune rabbit serum (O—O) incubation time 4 hours.

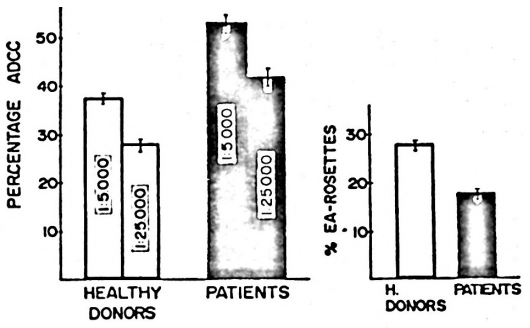

Fig. 2. *EA rosettes and antibody dependent cells cytotoxicity in normal individual and cancer patients.*
The cytotoxic activity (ADCC) of peripheral blood lymphocytes from normal individuals and cancer patients was 37.4 and 53.3 respectively when RACA (rabbit anti chicken antiserum) was used at 5×10^{-3} dilution. The same pattern e.g. more cytotoxicity in cancer patients than controls was obtained when RACA was used at 2.5×10^{-4} (28.3 and 42.3 %). The percentage of EA rosettes was determined in parallel with the following results: Normal individuals 28.2 %, cancer patients 18.7 %.

Table I. *EA-rosettes and ADCC in normal individuals.*

% of EA rosettes	% of ADCC	
	Dilution of RACA 5×10^{-3}	Dilution of RACA 2.5×10^{-4}
32	40.8±0.9	31.6±0.8
26	25.8±0.6	16.6±0.5
28	24.7±0.7	17.2±0.5
35	42.9±0.5	32.9±0.8
26	25.9±0.8	17.3±0.9
36	51.9±0.5	44.2±0.4
38	52.3±0.5	41.6±0.7
30	43.6±0.7	25.5±0.9
31	39.6±1.0	28.2±0.8
32	43.1±0.7	35.5±0.6
33	49.0±0.9	43.5±0.7
31	48.4±0.7	36.0±0.5
27	27.8±0.9	16.1±0.5
30	45.0±0.1	36.9±0.6
34	39.9±0.8	32.2±0.5
32	38.1±0.5	26.6±0.7
26	30.9±0.5	16.7±0.1
22	21.8±0.4	13.9±0.4
34	51.2±0.5	42.0±0.8
21	26.3±0.5	12.4±0.8

Fc receptor bearing cells (EA rosette) and antibody dependent cell cytotoxicity in normal individuals and cancer patients. The cytotoxic activity (ADCC) of PBL from cancer patients and normal individuals have been studied at two different concentrations of RACA (1 : 5,000 and 1:25,000). Fig. 2 shows in cancer patients the specific ^{51}Cr release was 53.3 % and 42.3 % respectively while in normal individuals was 37.4 and 28.3 %.

The percentage of EA rosetting cells was performed in parallel in the same blood samples (fig. 2). Cancer patients had a mean of 18.7 % Fc receptor bearing cells and normal individuals had one of 28 %. Individual results for ADCC and EA rosettes in both groups are recorded in table I and table II.

Statistical analysis of ADCC activity and Fc receptor bearing cells (EA rosetting cells) in both populations. The possible existence of a correlation between % EA rosettes and ADCC activity was investigated in controls (A) and cancer patients (B) when the dilution RACA 5×10^{-3} was used.

Fig. 3 shows the regression line of A

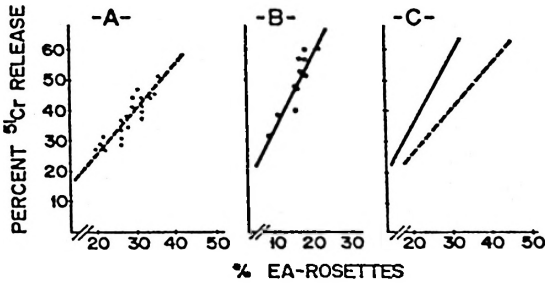

Fig. 3. *Relationship between % Fc receptor and ADCC activity.*
Dilution of RACA 5×10^{-3}. Incubation time 4 hours. A) Regresion line obtained in the control population ($r = 0.792$; $p < 0.01$). B) Regression line obtained in cancer patients ($r = 0.932$; $p < 0.01$). C) Comparison of both lines.

Table II. *EA-rosettes and ADCC in cancer patients.*

Tumours	% of EA-Rosettes	% of ADCC	
		Dilution of RACA 5×10^{-3}	Dilution of RACA 2.5×10^{-4}
Hodkin's disease	19	51.3±0.9	43.3±0.5
Leukaemia	17	44.3±1.0	35.9±0.3
Bronchial carcinoma	22	59.2±0.9	56.7±0.6
Mammary carcinoma	19	55.5±0.7	50.2±0.9
Stomach cancer	19	63.1±0.5	50.2±0.8
Melanoma	16	32.9±0.7	20.1±0.5
Bronchial carcinoma	18	46.9±0.7	32.1±0.6
Liver carcinoma	16	44.8±0.7	32.7±0.9
Mammary carcinoma	23	66.1±0.8	51.7±1.0
Mammary carcinoma	25	69.4±0.6	55.6±0.8
Mammary carcinoma	18	59.3±0.5	52.8±1.0
Squamous carcinoma	27	66.3±0.8	55.5±0.9
Cervix carcinoma	7	28.3±0.9	18.2±0.5
Breast carcinoma	12	37.9±0.5	27.6±0.7

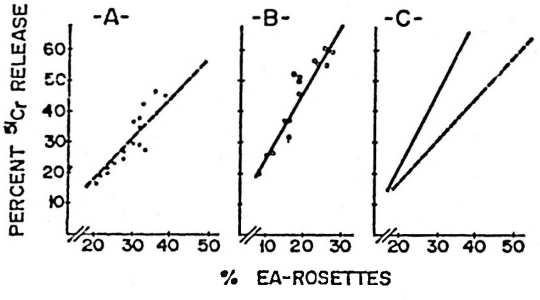

Fig. 4. *Relationship between ADCC and Fc receptors.*
Dilution of RACA 2.5×10^{-4}. Incubation time 4 hours. A) Regression line of the control population ($r = 0.830$; $p < 0.01$). B) Regression line obtained in cancer patients ($r = 0.932$; $p < 0.01$). C) Comparison of regression lines.

and B groups with a correlation r: 0.792, $p < 0.01$ (A) and r: 0.932, $p < 0.01$ (B). Therefore there was a relationship between Fc receptors and ADCC activity in both groups; however, when both groups were compared by using the Snedecor test (fig. 3C) their difference was made manifest. The same result was obtained when the mathematical analysis was done with the dilution of RACA 2.5×10^{-4} (fig. 4).

Discussion

The mechanism of lysis of antibody coated target cells by non immune lymphoid cells is not well understood. It has been clearly demonstrated in previous studies that ADCC against any type of target cells requires the presence on the target cell of antibody with intact Fc regions which bind to Fc receptors in the effector cell surface (12, 14). It seems that not all the Fc receptor bearing cells are active in the lysis of antibody coated target cells. For instance, human lymph node lymphocytes from normal donors and cancer patients with 10 and 20% of Fc receptor bearing cells respectively, were unable to kill antibody coated target cells (11). Furthermore, comparative studies between ADCC and Fc receptor bearing cells have been done (6) in various rat lymphoid organs and have shown no correlation between the percentage of EA rosetting cells and ADCC, despite the fact that cytotoxic activity is mediated by a subpopulation of cells that form EA rosettes. The results of our study demonstrate that there is a relationship between EA rosette forming cells and ADCC. However the regression lines were different in both groups (fig. 3 and 4).

Interestingly, cancer patients, who have a lower EA rosette forming cells than controls showed higher cytotoxic activity. There are several alternative explanations of the nature of these enhanced cytotoxic functions. Leading possibilities include

either an increase in the number of circulating effector cells of normal activity or the presence of normal numbers of hyperfunctional cytotoxic cells. In our *in vitro* assay the ratio of target cells to effector cells was constant in control and patients, so it cannot account for differences in the number of *in vivo* circulating K cells. The second possibility could imply that lymphoid cells from cancer patients are precomitted to destroy target cells more easily than the control population.

A marked reduction in K cell cytotoxicity during therapy with cytostatic drugs in acute lymphocytic leukemia (4) and multiple myeloma (9) has been demonstrated. However our study was performed in patients before receiving chemotherapy and include solid tumours like mammary carcinoma. Hodgkins disease and malignant melanoma (table II).

Resumen

Se investiga la posible relación existente entre la capacidad que tienen los linfocitos de sangre periférica de formar rosetas EA y su funcionalidad citotóxica mediada por anticuerpos frente a hematíes de pollo marcados con Cr^{51}.

Estudiando una población de enfermos cancerosos, se observa que hay correlación entre las células formadoras de rosetas EA y su actividad citotóxica. Sin embargo, comparando estos datos con los controles, se observa que a pesar de tener un menor porcentaje de rosetas EA, la actividad citotóxica de estas células es mucho mayor.

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