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Active E Rosette-Forming Cells in the Peripheral Blood of Cancer Patients*

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Abstract—*The percentage and the absolute number of cells forming active and total E rosettes were determined in 107 patients with tumors of the lung or esophagus or glioblastomas. Although both the active and total E rosette forming cell subpopulations of T lymphocytes were decreased in cancer patients, the percentage of reduction of the active rosettes was more pronounced and paralleled the depression of other parameters of T-dependent immunity. Therefore, evaluation of the active T cell subpopulation may provide a rapid and useful test in monitoring the immune function of patients affected with neoplasias.*

INTRODUCTION

SINCE the initial observations of rosette formation between human blood lymphocytes and sheep red blood cells (SRBC) [1, 2] the assay procedures for this test have been modified. The reaction depends on many technical factors [3, 4], some of which are utilized to differentiate the various T lymphocyte subpopulations present in normal blood and during the course of various diseases. Rosettes which form immediately after centrifugation of a mixture of SRBC and lymphocytes are called 'active' rosettes. Rosettes that require incubation at 4°C for optimal formation are called 'total' rosettes [5]. Rosettes that do not dissociate before reaching 37°C are called 'stable' [6]. Rosettes having more than 36 SRBC are defined as 'giant' rosettes [7]. 'Suspension' rosettes are those which form when lymphocytes and SRBC are maintained in suspension by tube rocking [8].

Both clinical [9, 10] and experimental [11] studies suggest that the active rosette forming cells represent a subpopulation of peripheral T lymphocytes which are more actively involved in cellular immunity than the total E rosette forming cells.

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In the present study the absolute number and the percentage of active (A-RFC) and total rosette forming cells (T-RFC) were evaluated in the peripheral blood of 107 patients affected with various solid tumors (esophageal, lung, brain) so as clarify the importance of these markers in the diagnosis of immune processes.

MATERIALS AND METHODS

Patients

The lymphocytes of 107 patients affected with solid tumors were studied. The patients' ages ranged from 36 to 81 yr. The types of solid tumors were as follows: (1) 47 esophageal tumors (43 squamous cell, 4 adenocarcinomas); (2) 27 lung tumors (22 squamous cell, 3 undifferentiated, 2 adenocarcinomas); (3) 33 glioblastomas. None of these patients had undergone surgery, chemotherapy or radiotherapy. Twenty-five apparently healthy subjects were accepted as controls.

Lymphocyte separation

Lymphocytes were separated from heparinized peripheral blood by centrifugation on a Ficoll–Hypaque density gradient [12]. The cells were washed three times in Hanks' balanced salt solution and resuspended in TC medium 199 (Difco Laboratories, Detroit, U.S.A.).

Total rosette-forming cells (T-RFC)

The total rosette assay was performed according to the method of Stjernswärd *et al.* [13]. SRBC were stored at 4°C in Alsever's solution (1/1) and were used within 1 week of being drawn; they were washed three times with Hanks' solution immediately before use. Lymphocytes (1×10^6) were incubated with SRBC (lymphocyte: erythrocyte ratio, 1:80) for 10 min, centrifuged at 200 ***g*** for 5 min and incubated at 4°C overnight. On the following morning the pellet was gently resuspended and 200 lymphocytes were counted in each sample; all determinations were performed in duplicate. Rosette-forming lymphocytes (T-RFC) were defined as those which bound three or more SRBC.

Active rosette forming cells (A-RFC)

A modification of the method of Wybran and Fudenberg [16], which in part had already been proposed by Smith *et al.* [15], was used as a test for active rosettes. This procedure eliminates the requirement of fetal calf serum (FCS) and incubation of lymphocytes before assaying for rosettes. One quarter of a ml (1×10^6) of lymphocytes were mixed with SRBC (lymphocyte: erythrocyte ratio, 1:20), incubated for 5 min at 37°C and centrifuged for 5 min at 200 ***g***. Resuspension and counting of A-RFC were performed as for the total rosettes. Using this modified procedure, we found that the percentage of A-RFC of the controls were comparable to the values obtained by the method of Wybran and Fudenberg [14, 16].

The absolute numbers of A-RFC and T-RFC were determined by calculation of the corresponding total and differential leukocyte counts. The results are reported as the mean percentage or absolute number/mm^3 of RFC ± S.E. Statistical differences were determined by Student's *t*-test.

The data for each patient were compared with the results of the *in vitro* blastic response which were determined by a method previously described [17].

RESULTS

Table 1 shows the percentage and absolute numbers of A-RFC found in the various solid tumors compared with those of the controls. The mean percentage and the absolute number of T-RFC in the same patients are summarized in Table 2. The values of both A-RFC and T-RFC were found to be markedly less than control values ($P < 0.001$). The results of total and active rosettes were evaluated in terms of percentage of reduction as compared with healthy subjects. As shown in Fig. 1, the reduction percentage of A-RFC was markedly greater than that of the T-RFC

Table 1. Percentage and absolute number of A-RFC in solid tumors

	Number	Percentage	Absolute number/mm^3	*P* value
Controls	25	22.8 ± 0.9	572 ± 49	—
Carcinoma of the esophagus	47	15.9 ± 0.7	378 ± 37	<0.001
Carcinoma of the lung	27	14.9 ± 1.1	293 ± 26	<0.001
Glioblastomas	33	16.2 ± 1.2	307 ± 28	<0.001

Table 2. Percentage and absolute number of T-RFC in solid tumors

	Number	Percentage	Absolute number/mm^3	*P* value
Controls	25	58.6 ± 2.1	1468 ± 121	—
Carcinoma of the esophagus	47	50.5 ± 1.0	1093 ± 92	<0.001
Carcinoma of the lung	27	49.2 ± 1.2	1148 ± 81	<0.001
Glioblastomas	33	50.8 ± 1.9	951 ± 64	<0.005

in all three groups of patients studied (percentage reduction: lung carcinoma, A-RFC 34%—T-RFC 16%; esophageal carcinoma, A-RFC 31%—T-RFC 13%; glioblastomas, A-RFC 30%—T-RFC 15%). These reduction percentages were then compared with other parameters of T immunity which were simultaneously carried out on the same patients. A significant test was *in vitro* lymphocytic blastigenesis which showed a depression of the blastic response in the majority of these patients. This depression was calculated as a percentage of reduction with respect to normal and was compared with the reduction percentage of the A- and T-RFC (Fig.1). The percentage of reduction of the blastic response resembled the reduction percentage of A-RFC more than the T-RFC in all three cases of solid tumors under study (percentage reduction of PHA induced blastigenesis: lung 39%, esophagus 32%, glioblastomas 37%).

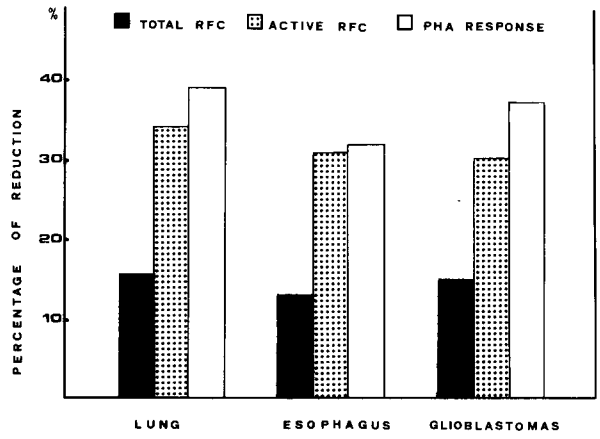

Fig. 1. Reduction percentage with respect to controls of active- and total-E-rosette-forming-cells compared with percentage of reduction of the blastic response to PHA in cancer patients.

DISCUSSION

In cancer patients the number of active E rosettes is markedly reduced in percentage, as well as in absolute number. This was found in almost all the cases with carcinoma of the lung or esophagus or glioblastomas under observation (untreated and newly diagnosed patients). The values of the T-RFC were also reduced, but the reduction was less pronounced. In fact, the percentage of reduction of T-RFC were approximately half those of A-RFC (Fig. 1).

An important parameter of T cell function is still the evaluation of blastigenesis induced by phytohemagglutinin. The percentage of reduction of the response to PHA overlapped with the percentage of reduction of A-RFC. On the other hand, the percentage of reduction of the T-RFC was considerably inferior to the reduction percentages of A-RFC or the PHA response (Fig. 1).

In our previous studies, a net increase in the formation of active E rosettes by PHA stimulated lymphocytes as compared to unstimulated lymphocytes has been demonstrated [11]. We suggested that the active E rosette forming cells constitute a specific subpopulation of lymphocytes which represent functionally active T cells in the blood. Another possibility, which does not exclude the former, is that the A-RFC may be due to the presence of immature T cell subpopulations which are leaving the thymus to populate peripheral lymphoid tissue. Horowitz *et al.* [10] suggested that the active RFC serve as a marker of T cell differentiation. Wybran *et al.* [5] demonstrated an elevated number of A-RFC in fetal thymuses and an increase in active E rosette formation after incubating normal lymphocytes with thymosine [18].

Human cancers have been known to be associated with various degrees of altered cell mediated immunity and the immunologic status of patients with carcinoma is a decisive factor in the behavior of the disease [19].

The metastatic spread of the neoplastic process and a low percentage of A-RFC may be correlated. Only few patients have been studied under this aspect. At the initial diagnostic encounter, these cases already showed a low average percentage of A-RFC (13.2%) relative to the values of other patients.

Active T cells may identify a clinically significant subpopulation of T lymphocytes [5, 10, 14]. Our results support the hypothesis that the A-RFC correlate better than total rosette formation with *in vitro* T cell function. Evaluation of this subpopulation provides a rapid and useful assessment for monitoring T cell function in human cancer.

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