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Association of Electrophoretic Mobility with other Cell Surface Markers of T Cell Subpopulations in Normal Individuals and Cancer Patients

E. E. UZGIRIS,* J. H. KAPLAN,* T. J. CUNNINGHAM,† S. H. LOCKWOOD* and D. STEINER†

*General Electric Corporate Research and Development, Schenectady, New York 12301, U.S.A.
†Albany Regional Cancer Center, Albany, New York 12208, U.S.A.

Abstract—*Two major electrophoretically distinct T cell components were observed in peripheral blood of normal donors. The cells comprising these components differed in their binding capacity for sheep red blood cells (SRBC). High-affinity E-rosette-forming cells (E-RFC) were obtained by rosetting at 29°C with limited numbers of SRBC and belonged to the high-mobility component. Low-affinity E-RFC belonged to the low-mobility component. In cancer patients the fraction of cells falling in the high-mobility class decreased by 20 ±11% of total cells, but no significant decrease of high-affinity E-RFC was observed. Thus, the correspondence between high-affinity and high-mobility found for normal donor lymphocytes may not hold for a subset of lymphocytes found in individuals with cancer.*

INTRODUCTION

THE STUDY of lymphocyte subpopulations is finding increasing acceptance in the evaluation of the immune status of patients in various disease states including cancer. The primary method of lymphocyte classification has consisted in identifying specific cell surface markers. The membrane receptor for sheep red blood cells (SRBC) is one example of a widely used surface marker that distinguishes T (thymus-derived) from B (thymus-independent) cells. Although T cells bind to SRBC and B cells do not, the strength of binding is not the same for all T cells. Recently West *et al.* [1] by varying the temperature and SRBC to lymphocyte ratio, demonstrated differing binding capacities of T cells for SRBC. Operationally, T cells were divided into two fractions: (1) "high-affinity" T cells that formed rosettes at 29°C with limited numbers of SRBC; and (2) "low-affinity" T cells that required incubation at lower temperatures (e.g., 4°C) with larger SRBC to lymphocyte ratios. This variation in binding strength has been used to show that the number of high-affinity T cells decreases in individuals with cancer and certain other diseases [2–4] while the total T cell population is not significantly affected in most of these patients.

Since rosetting of high-affinity T cells depends on the rather delicate differences in binding to SRBC as a function of temperature, it would be desirable to find another cell surface parameter which would detect different T cell subpopulations and their variations with disease. Specifically, we endeavored to answer the following two questions:

(1) To what extent does the discrimination of T cell subpopulations by electrophoretic mobility correspond to that made on the basis of binding affinity for SRBC? and (2). In individuals with cancer, are there changes in T cell mobility distribution that parallel the reported changes in distribution of SRBC-binding affinity?.

MATERIALS AND METHODS

Donor selection

Blood was drawn from normal volunteers (mean age 48) and from pre and post-operative cancer patients (mean age 54) receiving no therapy. A minimum of 3 weeks was allowed to elapse subsequent to surgery before studying

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the post-operative patients. Except for three individuals, all patients had either carcinoma of the breast or colon.

Mononuclear cell preparations

Mononuclear cells were isolated from whole heparinized blood by the gradient method of Boyum [5] using Ficoll–Hypaque. Cell viability, which was determined by exclusion of trypan blue, was 95% or better.

Cell surface marker determinations. Rosetting procedure for T cells

The procedure of West *et al.* [1] was used to identify and isolate total rosettes and high- and low-affinity E rosette-forming cells. Briefly, lymphocytes were incubated with SRBC (SRBC/lymphocyte ratio of 50/1) for 3 hr at 29°C to isolate high-affinity rosettes. To isolate total rosettes, lymphocytes were incubated with SRBC (SRBC/lymphocyte ratio of 150/1) at 4°C. To obtain low-affinity rosettes, cells not forming rosettes at 29°C were further incubated with SRBC (150/1) but at 4°C. Rosettes were isolated from nonrosetted cells by density gradient centrifugation on Ficoll–Hypaque. In order to recover the rosette-forming lymphocytes, the rosettes were broken apart by mechanical agitation at 37°C and the lymphocytes were separated from SRBC by centrifugation on a Ficoll–Hypaque gradient.

Electrophoretic mobility measurements

Electrophoretic mobilities were determined with the laser Doppler spectrometer [6] using methods previously described in detail [7,9]. The technique relies on the fact that a moving object will scatter light that is Doppler-shifted in frequency in proportion to its velocity. Hence, determination of scattered light spectra gives a velocity or mobility profile of the objects under study [7,10].

RESULTS

Laser Doppler spectra of normal human peripheral blood lymphocytes showed two large components (Fig. 1a). Surface marker studies [9] have shown that the two major spectral components, centered at 2.15 and 2.35 μm/sec/V/cm, respectively, are due to different T cell subpopulations, while the minor component below 2.0 μm/sec/V/cm is composed mainly of B cells. In contrast, post-operative cancer patients (Fig. 1b) showed a decrease of high-mobility (2.3–2.59 μm/sec/V/cm) T cells compared to what was observed in normal donors.

For determining the binding properties of lymphocytes to SRBC we chose the rosetting procedure of West *et al.* [1]. Using their conditions for high-affinity rosetting (29°C, SRBC to lymphocyte ratio of 50:1), we found that $35 \pm 10\%$ of the mononuclear cells of normal donors formed rosettes. When these rosettes were broken apart by mechanical agitation at 37°C we found that the recovered lymphocytes were of the high-mobility class (range of 2.3–2.59 μm/sec/V/cm). The cells that did not form rosettes were reincubated with SRBC at 4°C and the lymphocytes from these rosettes were isolated in the same way as above. These low-affinity cells were the slow T cell type, in the range of 2.0–2.29 μm/sec/V/cm. The total SRBC rosetting cell population determined under optimal rosetting conditions ($60 \pm 8\%$ of the cells) had both mobility subpopulations present. For details of the correspondence between cell mobility and E-RFC affinity the reader is referred to an earlier study [11]. Furthermore by fractionating lymphocyte subpopulations using monolayers of IgG-sensitized sheep or chicken erythrocytes, we were able to show, that for normal donors, the properties of high-mobility and high-affinity are associated with the same T cell subpopulation. [11].

In Table 1 we summarize the results for the first three experimental series in which we compared the mobility distributions of post-operative cancer patients and normal donors. To avoid systematic differences in each series one particular electrode was used each time and the experiments were mostly done in pairs for identical conditions of temperature and electric field. There was a reduction of the fast T cell subpopulation for the post-operative cancer patients relative to the normal donors by 20, 28 and 23% of total cells in each series, respectively. Figure 2 shows a histogram of mobility populations for the two types of donors taken from the last experimental series in which improved experimental procedures were used (local pH in the space between the electrodes was stabilized and cell measurements were done immediately after isolation). The fractions of cells in each of the three mobility bins for each donor are presented in Fig. 3.

Experimental series IV was undertaken to examine both cell mobility and SRBC-binding affinity of cancer patient lymphocytes relative to normal donor lymphocytes. The cell mobility in this series was measured in Dulbecco's phophate buffered saline (without Ca^{2+} and MG^{2-}) rather

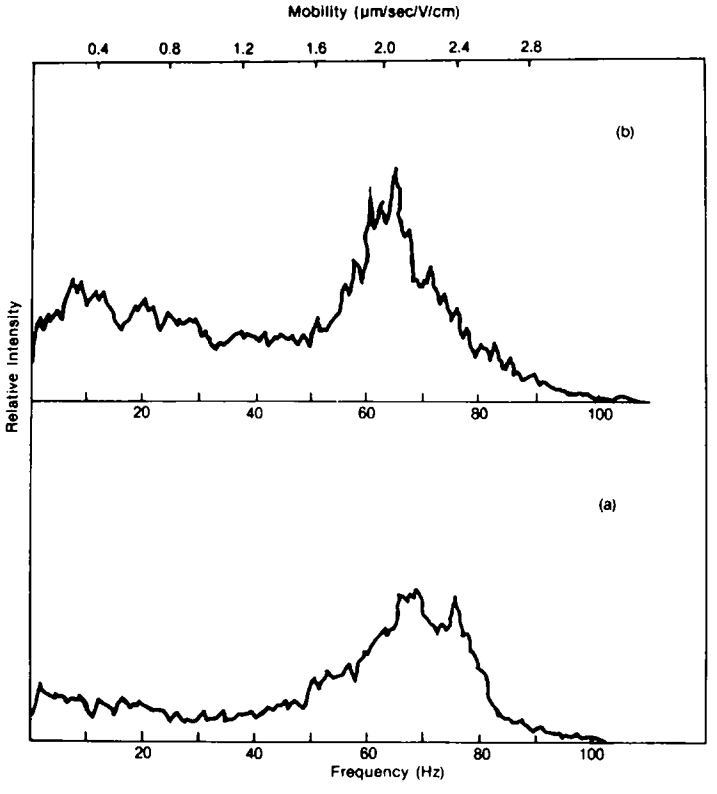

Fig. 1. Doppler spectra of mononuclear peripheral blood cells from a normal donor (a) and a post-operative cancer patient (b). The scattering used was typically 20° and the applied electric field was approximately 50V/cm. Usually 1×10^6 cells/ml were measured. The mobility values are given for 25°C in 0.28 M sucrose measuring medium of pH 7.3 made up by diluting 1 part of Seligmann's balanced salt solution with 29 parts of 0.29 M sucrose. The harmonic structure that appears in the spectra, denoted by regular intervals of frequency, is due to the switching of the electric field between opposite polarity at regular intervals in time.

Table 1. Lymphocyte mobility distributions in 0.005 ionic strength medium for normal and post-operative cancer donors

Experimental series	Donors	Per cent of cells: Slow (1.7–1.99 μm/sec/V/cm)	Intermediate (2.0–2.29 μm/sec/V/cm)	Fast (2.3–2.59 μm/sec/V/cm)
I‡	Normal (8)*	23±5†	46±10	29±11
	Cancer (9)	40±19	51±11	9±10
II‡	Normal (5)	30±8	30±2	38±7
	Cancer (8)	42±14	48±15	10±10
III	Normal (7)	16±9	43±10	40±12
	Cancer (8)	22±10	59±5	17±7

*Number in parenthesis indicates number of donors studied.

†Mean ± S. D.

‡Procedures in the first two series involved a 2–3 hr delay between time of cell isolation and time of measurement. The electrodes used in these measurements were subsequently found to induce occasional pH decrease near their surfaces. Both circumstances can result in an apparent depression of cell electrophoretic mobility and hence an apparent increase in the proportion of cells falling in the slow and intermediate mobility ranges.

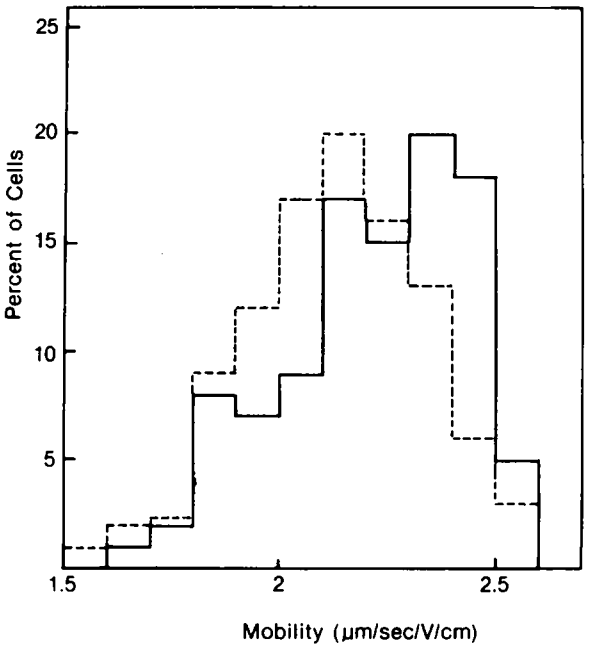

Fig. 2. Histogram of mobility distribution of mononuclear cells from 7 normal donors (———) and 8 post-operative cancer patients (- - - -). Local pH *in the space between the electrodes was verified to be stable with methyl red indicator dye and measurements of mobility were done within* 1 hr *of cell isolation.*

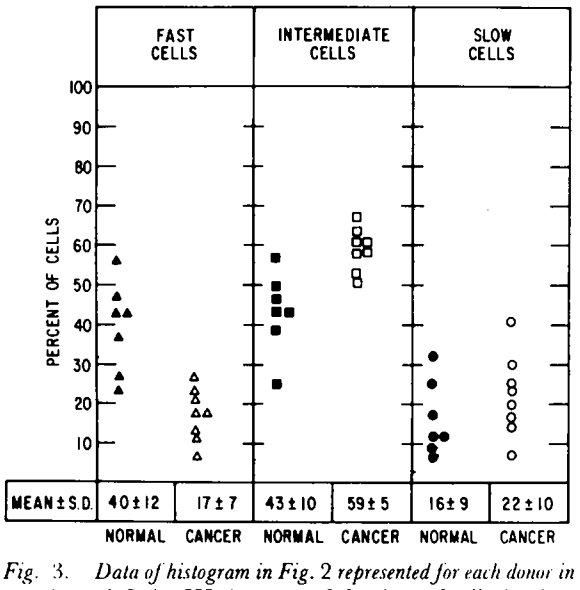

Fig. 3. Data of histogram in Fig. 2 represented for each donor in experimental Series III *in terms of fractions of cells in slow, intermediate, and fast mobility compartments.*

than dilute salt as before. This was done to utilize a newly developed capability for obtaining such measurements in high salt concentrations and also to verify that the earlier differences in mobility between cancer patients and normal donor lymphocytes could be extended to physiological salt conditions. Figure 4 shows Doppler spectra for a normal and pre-operative cancer patient. There is evidently a decrease of high-mobility cells for the cancer patient relative to the normal. Figure 5 summarizes the mobility and rosetting measurements for all the donors in the series. Here, for comparison purposes a fast mobility component was defined as the mobility equal to or in excess of 0.97 μm/sec/V/cm (fresh human red blood cells were used as markers of mobility in each experiment with their mean mobility value taken as 1.08 μm/sec/V/cm).

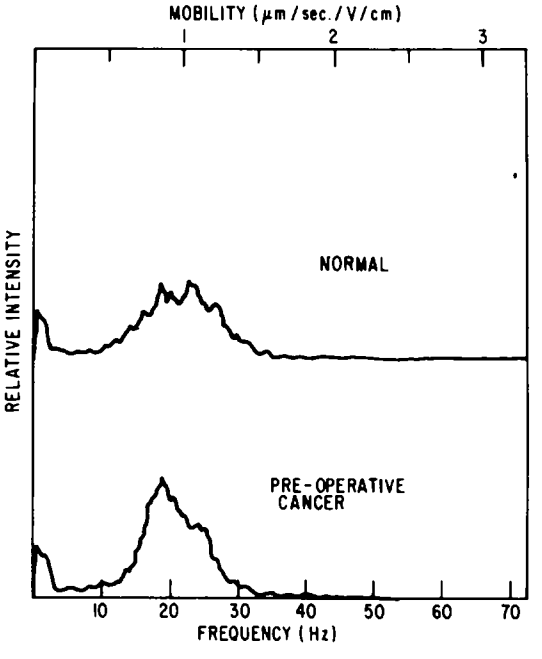

Fig. 4. Doppler spectra for normal and pre-operative cancer patient lymphocytes in phosphate buffered saline. Spectra taken for identical conditions of field, temperature, and scattering angle. Mobility scale calibrated by measurement of fresh human red blood cells whose mobility at 25°C *in saline is taken to be* 1.08 μm/sec/V/cm.

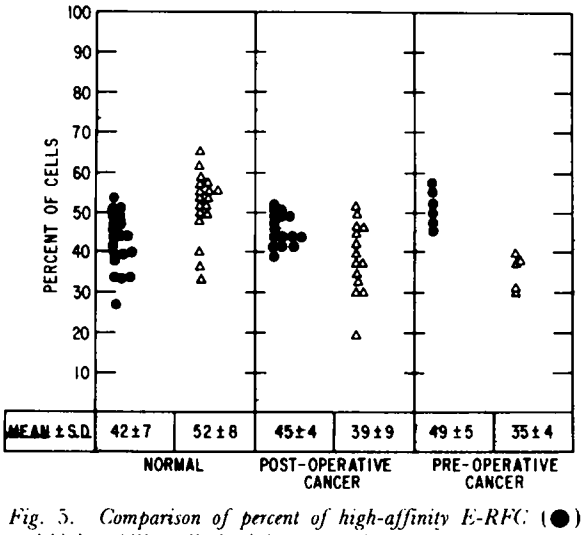

Fig. 5. Comparison of percent of high-affinity E-RFC (●) and high-mobility cells (△) in normal donors and pre- and post-operative cancer patients in experimental Series IV. *Mean values and standard deviation are indicated for each group of measurements.*

There is no significant change in the fraction of lymphocytes that form high-affinity E-RFC for either post-operative or pre-operative cancer patients relative to normals. However, the high-mobility component of these patients is significantly smaller than for the normal donors. This is similar to what was found with the earlier measurements in Series I-III in dilute salt.

The high-affinity E-RFC yield for normals was $42 \pm 7\%$ in this experimental series. For comparison West *et al.* [1] reported a yield of $54 \pm 5\%$ for normal donors.

DISCUSSION

Although we observed in this study a decrease in fast cells in cancer patients relative to normals, we did not find a significant depletion of high-affinity E-RFC as did West *et al.* [2] who found that relative to normal donors there was a depletion of high-affinity cells by approximately 20% of total cells in cancer patients with solid tumors including carcinoma of the breast, lung, colon, stomach and pancreas. This decrease occurred not only in patients with local disease and those with detectable metastases, but also in post-operative cancer patients with or without evidence of recurrent disease [3]. It is possible that, in our laboratory, the sensitivity for detecting small changes in affinity was not achieved and the differences between diseased and normal individuals failed to appear for that reason alone. Although this argument may seem plausible in view of the smaller yields of high affinity E-RFC that we obtained from normal donors ($42 \pm 7\%$) than were reported by West *et al.* ($54 \pm 5\%$), it is probably not correct. Further work [12] has shown that the incidence of donors with significantly depressed fractions of high-affinity E-RFC has dropped to a rather modest value for cancer patients, particularly for patients with carcinoma of the breast (29%). In other words, upon further examination, cancer patient lymphocytes appear to be similar to normal donor lymphocytes in the property of high-affinity binding to SRBC. From this recent observation and from our own work we infer that for breast and colon cancer patients there either is no change in the fraction of high-affinity E-RFC or the change is too difficult to detect due to small but perhaps important variations in experimental procedure.

As noted above, we obtained consistently smaller fractions of high-affinity E-RFC than did West *et al.* ($42 \pm 7\%$ compared to $54 \pm 5\%$) for normal donors. One possible reason for this difference may be due to our use of varying lots of SRBC that were usually more than 1 week old. By contrast, in their different experiments, West *et al.* [1,2] used SRBC drawn within 1 week from the same individual animal.

Our work is in agreement with an earlier observation of Plagne *et al.* [13] who showed a decrease in the fraction of rapid-migrating lymphocytes in the peripheral blood of cancer patients relative to normal donors. These workers identified these lymphocytes as T cells [14,15] but did not further associate them with a particular T cell subpopulation.

For normal donors our association of high-mobility with high-affinity E-RFC has been recently verified by Platsoucas *et al.* [16]. Using a density gradient electrophoresis method they found that the high-mobility fractions also exhibited the property of high-affinity binding to SRBC.

The association of two characteristics of cell surface, i.e., high-mobility and high-affinity binding to SRBC, appears to hold for normal donors but not for cancer patients. In these patients there is a subset of T cells, 15–20% of peripheral blood lymphocytes, that is of reduced electrophoretic mobility but which appears to be capable of binding to SBRC at 29°C. The lack of correspondence between high cell surface charge and high-affinity binding to SRBC for this subset of cells should be examined further. In particular, it would be instructive to examine these cells with other cell surface markers such as Fc receptors for IgG and IgM [16].

There have been recent reports [17, 18] showing changes in the percentage of "active" T cells [19] in cancer patients but there also have been other reports [20, 21] to the contrary. Apparently, the procedure of rosetting under suboptimal conditions depends on many factors and may be difficult to standardize [4, 22] as this study shows. On the other hand, the determination of electrophoretic mobility is a more direct measurement of cell surface properties. Therefore, cell mobility may be a more reliable parameter for the assessment of the immune status of cancer patients.

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