DIFFERENCES BETWEEN LEUKOCYTE MEMBRANES DETECTABLE BY A FLUORESCENT PROBE IN CHRONIC LEUKEMIAS

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Leukocytes of patients with various forms of leukemia have been for many years the object of intensive research by various techniques. As a result changes have been found in cell morphology and various immunologic receptors and changes in enzyme activity, chromosomal structure, and so on, have been found. These changes have served as the basis for methods of diagnosis of leukemias [4, 6]. The development of ideas and methods in the biophysics of cell membranes in recent years has led to the suggestion that disturbances of such physical properties of the membrane and its permeability, surface charge, and viscosity could take place in leukemic cells. If such disturbances do in fact take place, they must lead to changes in the concentration of substances in different parts of the cell, in activity of the membrane enzymes and, as a result, they must cause disturbances of the cell morphology.

Accordingly an attempt was made to discover differences between membranes of leukemic cells and normal lymphocytes by means of the method of membrane fluorescent probes. Previously when this method was used differences were found in membranes of human T and B lymphocytes, changes were found in the lymphocytes in bronchial asthma [1], and changes in the viscosity of membranes were discovered in cultures of leukemic cells and, in some cases, in cell membranes from patients with chronic lymphatic leukemia [8, 9].

EXPERIMENTAL METHOD

Cells from five patients with chronic lymphatic leukemia and six patients with chronic myeloid leukemia were tested. Twelve healthy donors formed the control group. Packed white cells were obtained during the procedure of plasmacytopheresis by means of an Aminco blood cell separator [3]. The cells were additionally purified in a Ficoll-Urografin density gradient [7]. In material taken from healthy blood donors and patients with chronic lymphatic leukemia the fraction at the partition boundary between the zones contained mainly lymphocytes, whereas in material taken from patients with chronic myeloid leukemia it contained many cells of the granulocyte series (with different degrees of maturity). Staining of the cells by the fluorescent probe 3-methoxybenzanthrone (MBA) and the method of measuring their fluorescence were described previously [2].

The histograms of the fluorescent cells were analyzed mathematically by the gradient descent method, as described in [5], on the M-4030 computer.

EXPERIMENTAL RESULTS

After the leukocytes had been stained with the MBA probe, they differed from each other in the intensity of their fluorescence. Histograms of the fluorescent cells are illustrated in Fig. 1. The mean intensity of fluorescence of the lymphocytes of patients with chronic lymphatic leukemia was rather higher than that of the healthy donors, but the level of significance of these differences was low ($P > 0.2$). Conversely, cells from patients with chronic myeloid leukemia had fluorescence on average 2.2 times brighter than the blood donors' lympho-

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TABLE 1. Parameters of Fluorescence of Cells Stained with MBA Probe in Patients with Chronic Leukemias and Blood Donors (M ± m)

Group tested	Number tested	Number of changes	Mean intensity of fluorescence, relative units
Healthy donors	12	12	32,0±1,2
Patients with chronic lymphatic leukemia	5	8	34,0±1,2
Patients with chronic myeloid leukemia	6	9	71,0±3,8

TABLE 2. Changes in Parameters of Fluorescence of Cells Stained with MBA Probe in Patients with Chronic Leukemias and Blood Donors (M ± m)

Group tested	Parameter				
	M_1	σ_1	M_2	σ_2	N_2/N_1
Healthy donors	27,0±1,2	7,0±0,5	43,0±1,3	11,0±1,1	0,59
Patients with chronic lymphatic leukemia (2)	30,0±1,6	6,0±0,4	40,0±1,4	9,0±0,8	0,62
P_{2-1}	<0,07	<0,25	<0,15	<0,35	<0,85
P_{2-3}	<0,001	<0,001	<0,001	<0,05	0,25
Patients with chronic myeloid leukemia (3)	59±3,1	15±1,5	92±7,4	15±2,2	0,87
P_{3-1}	<0,001	<0,001	<0,001	<0,15	<0,20

cytes. Bright fluorescence of the cells was found in all patients with myeloid leukemia (Table 1).

The intensity of fluorescence in three patients with myeloid leukemia fell by 10-30% after repeated cytopheresis (5-13 days after the first), but it still remained significantly higher than the level in the blood donors.

Since the mean intensity of fluorescence was found to be insensitive to changes in the lymphocytes taking place in chronic lymphatic leukemia, an attempt was made to discover whether the shape of the histogram was more sensitive to this leukemia. For this purpose each histogram was represented as the sum of two curves, each described by a normal gaussian distribution (Fig. 1, broken lines). Analysis showed that the sum of the two curves described the shape of the real histogram well; one curve was insufficient to describe the shape, and three curves were no better than two. The real histogram for each person in that case was described by six parameters: the position of the maxima of the two gaussian curves (M_1 and M_2), the width of the curves (δ_1 and δ_2), and the number of cells belonging to each curve (N_1 and N_2)

Comparison of the parameters mentioned above showed (Table 2) that the values of M_1 and M_2, and also the ratio of bright to dull cells (N_2/N_1) were higher in chronic lymphatic leukemia than the corresponding parameters determined by the study of lymphocytes from blood donors, although the level of significance of the differences was low. Meanwhile in chronic myeloid leukemia the cells already differed significantly from lymphocytes of blood donors and patients with chronic lymphatic leukemia with respect to the parameters M_1, M_2, and δ_1. It was also found that the N_2/N_1 ratio was increased in 70% of all patients with chronic leukemias tested compared with the mean value of this parameter in the group of healthy donors.

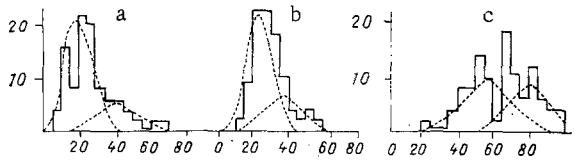

Fig. 1. Histograms of distribution of leukocytes by fluorescence of MBA probe. a) Lymphocytes of healthy blood donor, b) lymphocytes of patient with chronic lymphatic leukemia, c) cells from patient with chronic myeloid leukemia. Abscissa, intensity of fluorescence of cells (in relative units); ordinate, number of cells with the given level of fluorescence (in % of total number of cells studied from the same person).

Recording fluorescence of peripheral blood leukocytes stained by the MBA fluorescent probe thus provides an objective method (by quantitative measurements) of identifying differences between the membranes of normal lymphocytes and the membranes of lymphocytes in chronic lymphatic leukemia and also of cells in chronic myeloid leukemia. The physical basis of these differences has so far not been studied; evidently in the cases examined above the cells differed either in the number of membranes or in their physiological structure. In particular, in chronic myeloid leukemia differences in fluorescence of the membranes may be due to the presence of cells of the granulocyte series in the fraction, which normally contains mainly lymphocytes.

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