Acta Paediatrica Hungarica 30 (2) pp 291-302 (1990)

# CELL MEMBRANE FLUIDITY IN BLAST CELLS OF CHILDREN WITH ACUTE LEUKAEMIA

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Received 8 February 1989

Plasma membrane fluidity has been investigated by determining steady-state fluorescence polarization (FP) of the apolar stain 1,6,-diphenyl-1,3,5,-hexatriene in intact blast cells, separated from peripheral blood and bone marrow of children with various types of acute leukaemia. FP-values of blast cells taken before antileukaemic therapy were compared with FP-values of peripheral blood and bone marrow mononuclear cells separated from patients in complete remission as well as from control patients and from healthy volunteers. Moreover, fluorescence polarization measurements were also performed using blast cells of leukaemic patients on short-term single-drug prednisolone pretreatment. The results have shown that untreated blast cells have significantly lower FP-values than normal mononuclear cells of peripheral blood or bone marrow. No compartment difference has been observed within blast cells, while normal mononuclear cells from peripheral blood have significantly higher FP-values than bone marrow cells. FP-values of cells separated in remission or during prednisolone treatment do not differ from control values.

## INTRODUCTION

It is widely accepted that development of tumours evolves in several steps. The mechanisms by which transformed cells escape the control of physiologic extracellular signals are particularly important. This type of communication between the cell and its environment takes place at the cytoplasmic membrane. Previous studies have suggested that plasma membrane lipid fluidity is of utmost significance in transmembrane

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signalling and transport /16/. Though the exact relationship between oncogenesis and membrane fluidity is not yet clear, microviscosity, a term characterising both structural and diffusion properties of the lipid compounds of the plasma membrane, is expected to play an important role in governing normal and abnormal growth and differentiation of haemopoietic cells /4-9, 12, 17, 18/. Moreover, surface membrane fluidity of tumour cells has been shown to be an important factor in determining their ability to resist to or to escape immune killing of humoral factors /10, 19/ or immunocompetent cells /11, 20/. Previous studies on normal and leukaemic cell populations have pointed to a correlation between membrane fluidity and activity of the disease /1/.

In this study, plasma membrane fluidity of blastic mononuclear cells, obtained from peripheral blood and from bone marrow of acute leukaemic children either before introduction of therapy or in remission, has been investigated and compared with mononuclear cells separated from healthy adult volunteers and from children with nonmalignant disorders. Plasma membrane fluidity was studied by using 1,6,-diphenyl-1,3,5,-hexatriene (DPH), one of the most current fluorescent membrane markers (15, 13).

## PATIENTS AND METHODS

### Patients

Peripheral blood and bone marrow were taken from 18 children with acute lymphoid leukaemia and from 7 children with acute myeloid leukaemia. In 17 cases, samples were taken both at the onset of the disease and during complete remission. In addition, 5 long-term survivors were included in the remission group. Diagnosis was based on panoptically stained smears as well as on cytochemical and immunological reactions. Peripheral blood samples of 10 patients on short-term single-drug prednisolone pretreatment on the second to fourth day of treatment were also studied.

The control group consisted of healthy young adult volunteers and children with nonmalignant disorders (immune thrombocytopenic purpura, infectious mononucleosis, other viral infections, pyelonephritis and recovery after transient neutropenia). In each case, bone marrow aspiration was performed to exclude a malignant disease. In these patients

bone marrow smears indicated normal or megakaryocytic haemopoiesis.

Cell separation

Mononuclear cells were separated from heparanized blood and bone marrow aspirate by Lymphoprep (Nycomed AS, Oslo, Norway) gradient centrifugation according to Boyum /3/, washed twice with phosphate buffered saline, pH 7.4 (PBS; Gibco) and diluted to the appropriate concentration. Viability of the cells was always over 95 % as detected by the trypan blue (Serva) exclusion method. Only fresh cells were used for the further procedures.

Labelling the cells

DPH (Sigma) was dissolved in tetrahydrofuran (Serva), $2 \times 10^{-3}$ mol/L. 0.1 mL of this DPH stock solution was further diluted in 100 mL PBS, applying vigorous stirring at room temperature. The clear DPH dispersion exhibited no fluorescence. A volume of 2.0 mL cell suspension in PBS was incubated with 2.0 mL DPH dispersion at 37 °C for 15 min. The labelled cells were then washed twice with PBS, resuspended in PBS at a concentration of approximately $5 \times 10^5$ cells/mL, to reduce bias due to scattered light. All samples were measured immediately after labelling.

Steady-state fluorescence polarization

Fluorescence was measured in a Hitachi MPF-4 fluorescence spectrophotometer equipped with polarizers of 360 nm excitation and 425 nm emission wavelengths. Measurements were performed in thermostated silica cells at 25 °C and at 37 °C.

Fluorescence polarization (FP) and fluorescence anisotropy (r) were calculated as follows:

$$FP = \frac{I_{VV} - GI_{VH}}{I_{VV} + GI_{VH}}, \qquad r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

where $I_{VV}$ and $I_{VH}$ stand for fluorescence intensities measured with vertical and horizontal analyzers, respectively, keeping the polarizer in vertical position, and G is the correction factor ($G = I_{HV}/I_{HH}$).

Microviscosity was calculated according to Perrin's equation:

$$\frac{r_0}{r} = 1 + C(r)\frac{T\tau}{\eta}$$

where $r_0 = 0.362$ stands for the rate limiting fluorescence anisotropy, r for calculated fluorescence anisotropy of the sample, $\eta$ is microviscosity of the medium in which DPH molecules are embedded, $C(r) = 8.5 \times 10^4$ Pascal x deg$^{-1}$ x sec$^{-1}$, is a parameter that relates to molecular shape of the fluorophore, T is absolute temperature, and $\tau$ is the excited state lifetime of DPH, as estimated directly from the temperature profile of fluorescence intensity assuming a limiting value, $\tau_0 = 11.4$ nsec at 0 °C, as determined by Shinitzky and Barenholz /13/. Fluorescence polarization (FP) is directly related to microvis-

cosity ($\eta$) and both exhibit an inverse relationship to surface membrane fluidity.

Data analysis

Mean and standard deviation (SD) values of FP were calculated. The level of statistical significance was estimated by Student's t-test.

## RESULTS

Steady-state fluorescence polarization of DPH labelled mononuclear cell suspensions separated from acute leukaemic children were studied at manifestation of the disease and during remission, as compared to that of control persons. The FP- and $\eta$-values, of blood cells of patients and of control persons are summarized in Table I. As shown in the table, there is no significant difference 1) between FP-values of the peripheral blood blast cells and of the bone marrow cells of leukaemic children; 2) between FP-values of the peripheral blood mononuclear cells separated from patients in remission and from control persons; and 3) between FP-values of bone marrow mononuclear cells of the latter two groups. Therefore, the corresponding data were collapsed and three groups were formed for further statistical analysis: 1) leukaemic blast cells, i.e. cells separated from peripheral blood and bone marrow of patients before therapy, 2) normal peripheral blood mononuclear cells, obtained from patients in remission and from control persons, and 3) normal bone marrow cells, obtained from patients in remission and from control persons. The fluorescence parameters of these three groups are shown in Table II. As it can be seen from the table, mean FP-values of leukaemic blast cells are significantly smaller than mean FP-values of normal mononuclear cells either of the peripheral blood or of the bone marrow, at 25 $^{o}$C and 37 $^{o}$C alike. Moreover, mean FP-values of normal peripheral blood mononuclear cells are significantly greater than the corresponding parameters of normal bone marrow mononuclear cells, both at 25 $^{o}$C and 37 $^{o}$C. Table III shows the FP-values of cells

TABLE I

Fluorescence polarization and membrane microviscosity ($\eta$) of DPH-labelled mononuclear (MN) cells, separated from peripheral blood or bone marrow of acute leukaemic children at the onset of the disease; the corresponding data obtained in control persons are also given

| | Temperature | | | | | |
|---|---|---|---|---|---|---|
| | 25 °C | | | 37 °C | | |
| Investigated cells (Number of cells) | Fluorescence polarization range | Fluorescence polarization mean $\pm$ SD | $\eta$ (Poise) mean | Fluorescence polarization range | Fluorescence polarization mean $\pm$ SD | $\eta$ (Poise) mean |
| Leukaemic blast cells, peripheral blood (24) | 0.160-0.254 | $0.207 \pm 0.020$[a] | 1.847 | 0.143-0.236 | $0.182 \pm 0.022$[d] | 1.375 |
| Leukaemic blast cells, bone marrow (21) | 0.166-0.244 | $0.204 \pm 0.019$[a] | 1.785 | 0.151-0.222 | $0.180 \pm 0.018$[d] | 1.342 |
| Peripheral blood MN cells of patients in remission (22) | 0.226-0.303 | $0.267 \pm 0.020$[b] | 3.084 | 0.209-0.283 | $0.238 \pm 0.018$[e] | 2.221 |
| Peripheral blood MN cells of control persons (37) | 0.243-0.295 | $0.266 \pm 0.015$[b] | 3.084 | 0.199-0.277 | $0.234 \pm 0.020$[e] | 2.149 |
| Bone marrow MN cells of patients in remission (12) | 0.225-0.280 | $0.251 \pm 0.016$[c] | 2.670 | 0.189-0.243 | $0.216 \pm 0.017$[f] | 1.837 |
| Bone marrow MN cells of control persons (13) | 0.226-0.277 | $0.250 \pm 0.015$[c] | 2.670 | 0.199-0.223 | $0.214 \pm 0.009$[f] | 1.817 |

[a-f] indicate that there is no significant difference between the corresponding groups

TABLE II

Fluorescence polarization and membrane microviscosity ($\eta$) of DPH-labelled leukaemic blast cells and normal mononuclear (MN) cells separated from peripheral blood and bone marrow

| Investigated cells (Number of cells) | 25 °C Fluorescence polarization range | 25 °C Fluorescence polarization mean $\pm$ SD | Temperature $\eta$ (Poise) mean | 37 °C Fluorescence polarization range | 37 °C Fluorescence polarization mean $\pm$ SD | $\eta$ (Poise) mean |
|---|---|---|---|---|---|---|
| Leukaemic blast cells (45) | 0.160-0.254 | $0.206 \pm 0.019^{a,b}$ | 1.826 | 0.143-0.236 | $0.181 \pm 0.020^{d,e}$ | 1.359 |
| Normal MN cells separated from the peripheral blood (59) | 0.226-0.303 | $0.266 \pm 0.017^{a,c}$ | 3.084 | 0.199-0.283 | $0.236 \pm 0.019^{d,f}$ | 2.173 |
| Normal MN cells separated from the bone marrow (25) | 0.225-0.295 | $0.251 \pm 0.015^{b,c}$ | 2.670 | 0.189-0.243 | $0.215 \pm 0.013^{e,f}$ | 1.817 |

$^{a-f}$ indicate satistically significant differences ($p < 0.001$) between the corresponding groups

separated from three particular patients. Samples 1 and 2 are

TABLE III

Fluorescence polarization of DPH-labelled mononuclear (MN) cells separated from three particular patients with acute lymphoid leukaemia

| Sample | Case | Fluorescence polarization at 25 °C | 37 °C |
|---|---|---|---|
| 1. Peripheral blood MN cells | isolated meningeal relapse | 0.274 | 0.242 |
| 2. Bone marrow MN cells | isolated meningeal relapse | 0.265 | 0.231 |
| 3. Peripheral blood MN cells | isolated bone relapse | 0.262 | 0.238 |
| 4. Peripheral blood MN cells | aleukaemic manifestation | 0.311 | 0.269 |

mononuclear cells isolated from peripheral blood and bone marrow a boy affected by isolated meningeal relapse complicating of acute lymphoid leukaemia. Sample 3 represents the peripheral blood mononuclear cell suspension of a girl with acute lymphoid leukaemia in isolated bone relapse. Sample 4 represents the peripheral blood mononuclear cell suspension of a boy affected by acute lymphoid leukaemia in relapse with an aleukaemic manifestation. The common feature of these samples was that they were free of atypical blast cells, as checked either by panoptical smears or by using monoclonal antibodies. These FP-values were similar to control values. However, cells of the infiltrated bone marrow of the patient with peripheral aleukia were characterized by low FP-values (0.192 and 0.175 at 25 °C and 37 °C, respectively).

10 patients with acute lymphoid leukaemia were given a short-term course of single-drug prednisolone pretreatment. Mononuclear cells were separated from the peripheral blood on

the second to fourth day of this treatment. The cell suspensions contained 65-80 % atypical blast cells at that time. Fluorescence polarization measurements were performed only at 37 °C because of the great decrease in peripheral blood blast cells due to treatment. As shown in Table IV , the mean FP-value of these cells is significantly higher than the mean of leukemic blast cells before therapy while it does not differ from the corresponding normal value.

TABLE IV

Increase in fluorescence polarization and in membrane microviscosity ($\eta$) during prednisolone pretreatment

| Investigated cells (Number of cases) | Temperature: 37 °C Fluorescence polarization mean $\pm$ SD | $\eta$(Poise) mean |
|---|---|---|
| Leukaemic blast cells during Prednisolone pretreatment (10) | 0.233 $\pm$ 0.010[a] | 2.149 |
| Leukaemic blast cells before cytotoxic treatment (45) | 0.181 $\pm$ 0.020[a] | 1.359 |
| Normal mononuclear cells of the peripheral blood (59) | 0.236 $\pm$ 0.019 | 2.173 |

[a] indicates statistically significant difference ($p < 0.001$)

## DISCUSSION

Since the original observation by Shinitzky and Inbar /15/ many other studies have also confirmed that leukaemic blast cells have a more fluid, i.e. less viscous plasma membrane than normal lymphocytes. However, data are somewhat controversial about the question which parameters characterize best membrane fluidity and what their possible importance is. Dissent can be ascribed to differences in the source of the leukaemic blast

cells: established cell lines and primary blast cells were separated from different compartments; from peripheral blood, bone marrow or lymph node. In addition, various separation methods were applied: intact cells or membrane fragments were studied. Further, various dyes and incubation times were used for labelling. Therefore, we decided to investigate steady-state fluorescence polarization of DPH-labelled, freshly isolated, intact blast cells separated from peripheral blood and bone marrow of acute leukaemic children. We determined and compared membrane fluidity parameters at the onset of disease, during short-term single-drug prednisolone pretreatment, and in remission. Particular care was taken on accuracy of labelling time in order to get information exclusively about the fluidity of the plasma membrane /2/. Measurements were performed both at 25 $^{o}$C and at 37 $^{o}$C because of the possibility that the different phase transition temperatures of the membrane lipids of the blast cells and of the normal mononuclear cells might influence the FP-values. In our study, the surface membrane of leukaemic blast cells has indeed proven to be more fluid than the plasma membrane of normal mononuclear cells of either peripheral blood or bone marrow. The results suggest that high membrane fluidity, i.e. low microviscosity, may be an inherent feature of leukaemic blast cells. This view is supported by the following facts: 1) Membrane fluidity parameters of the blast cells are not influenced by their origin. Mean FP-values of blast cells, separated form either peripheral blood or bone marrow, do not significantly differ. 2) The mean FP-value of leukaemic blast cells is significantly smaller than the mean control value. 3) Plasma membrane fluidity of mononuclear cells, obtained from peripheral blood or bone marrow of patients in remission does not differ from that of corresponding control cells. 4) Mononuclear cell suspensions containing no blast cells separated from leukaemic patients with manifest disease (samples 1-4 in Table III) exhibited FP-values as seen in normal mononuclear cells.

Lipid microviscosity of the plasma membrane has been reported to regulate the availability of membrane-associated proteins, like antigens and receptors, the affinity of

receptors, and the activity of membrane-bound enzymes. More complex events, such as capping, cell proliferation and killing are also dependent on membrane fluidity /4-9, 12, 17-19/. The high fluidity of leukaemic blast cells may therefore correspond to their overwhelming proliferation and maturation arrest. In this context it may be interesting that prednisolone treatment "corrects" membrane fluidity of the blast cells (Table IV). A similar observation has been published by Ben Basset et al. who reported an increase in FP-values during treatment in a single patient with acute lymphoid leukaemia /1/. Our results point to a possible role of DPH in early diagnosis of leukaemia and its relapses. However, this needs further confirmation.

By using mononuclear cells of control patients and healthy volunteers for reference, an interesting difference between membrane fluidity of peripheral blood and bone marrow cells emerged: bone marrow cells have a more fluid plasma membrane than peripheral blood cells. As far as we know, such an observation has not been reported yet. The difference may lie either in the cells themselves or in their compartment environment.

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