EFFECT OF ERYTHROCYTIC CHALONE ON ELECTROPHORETIC MOBILITY OF MOUSE BONE MARROW CELLS

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Five fractions were isolated by cell electrophoresis in a Ficoll density gradient from the bone marrow of mice receiving physiological saline (control) and erythrocytic chalone (experiment). The absolute and relative distribution of the cells in the control and experimental fractions differed: the maximal number of cells in the control (up to 78%) was found in fractions 3-5 and the minimal (up to 22%) in fractions 1-2. A redistribution of the cellular material in all the fractions took place 25 min after treatment with erythrocytic chalone. The number of cells in fraction 1 was particularly increased (by 2.6 times). Analysis of the "myelograms" of films from fraction 1 in the experimental and control series showed that the number of cells in fraction 1 was increased on account of cells of the erythroid series. Among the proliferating cells of the erythron, the greatest decrease in electrophoretic mobility was observed in the proerythroblasts. It is suggested that as a result of interaction between erythrocytic chalone and membrane receptors of proliferating cells of the erythron their electric charge is reduced and changes take place in intracellular processes leading to delay in mitosis.

KEY WORDS: erythrocytic chalone; cell electrophoresis; bone marrow cells.

Investigations have shown that special substances (chalones), which have a selective action on cells and inhibit highly important intracellular processes such as DNA and RNA synthesis and mitosis, can be isolated from various tissues and organs [8, 9].

During interaction between the chalone and cell, one of the primary processes ultimately leading to inhibition of proliferation is evidently a change in the surface charge of the membrane. If this is so, after administration of a chalone the electrokinetic properties of the cells should be modified.

Accordingly, in the present investigation the electrophoretic mobility of bone marrow cells was studied after the action of erythrocytic inhibitor (chalone).

EXPERIMENTAL METHOD

The inhibitor was obtained from erythrocytes of noninbred albino rats weighing 150-200 g (50 animals) by the method described in [10]. For this purpose the erythrocytes were separated from contaminating leukocytes by centrifugation and washed twice with cold Hanks' solution to remove anticoagulant and plasma proteins. The erythrocytes were then treated with Hanks' solution (3:1). The hematocrit index of the resulting "polycythemic" erythrocyte suspension was thus 75%. After incubation for 1 h at 37°C the erythrocyte suspension was centrifuged at 3,000 rpm for 20 min and the supernatant was collected and used as the inhibitor of erythropoiesis. The supernatant contained several fractions of proteins with an inhibitory action on erythropoiesis [10]. To inactivate the thermolabile erythrocytic G1 chalone the supernatant was heated to 37°C before use [3]. The relatively thermolabile chalone remaining in the supernatant is destroyed only at 60°C. The writer's observations have shown that this type of erythrocytic inhibitor belongs to the class of erythrocytic proteins with the electrophoretic mobility of a1-globulins in polyacrylamide gel [4]. Fraction A in the supernatant obtained after incubation of erythrocytes possessed this mobility (Fig. 1).

To determine the quantity of inhibitor in the supernatant the first step was to measure the total protein content spectrophotometrically (SF-16, λ = 280 nm). Bovine serum albumin was used as the standard. The supernatant was then fractionated by disk electrophoresis in polyacrylamide gel [2]. To identify the hemo-
Fig. 1. Densitogram after disk-electrophoresis of "polycythemic" incubation medium.
A) Protein fraction with electrophoretic mobility of \( \alpha_1 \)-globulins (prahemoglobin), B) hemo-
globin, C) posthemoglobin fraction. Conditions of electrophoresis: gel system No. 1, cur-
rent 1-1.8 mA per tube, fractionation time 3 h at 2°C. Stained with Coomassie.

Fig. 2. Electrophoresis of mouse bone marrow cells in Ficoll density gradient. 1-5) Bound-
daries for collection of separate fractions.

globin zones in the gel, a commercial Hb preparation (from Reanal, Hungary) also was subjected to electro-
phoresis. The conditions of electrophoresis were described in detail previously [4]. After densitometry (the
densitometer was from East Germany) of the gel after electrophoresis, the relative content of the proteins of
fraction A was determined gravimetrically and, knowing the total protein content in the supernatant, the abso-
lute quantity of inhibitor in it was calculated. The investigations showed that the quantity of inhibitor (Fig.
1A) in the "polycythemic" incubation medium was 2.95 mg/ml. The inhibitor was injected intraperitonea-
ly into noninbred albino mice in a dose of 3 mg/ml. Physiological saline was injected into the control animals.
Altogether 46 mice weighing 20-22 g were used. The inhibitory action of the erythrocytic chalone was es-
timated 4 h after injection by determining the stahmokinetic index among the proliferating cells of the erythron
[5].

Some of the animals were killed 25 and 45 min after injection of the inhibitor and physiological saline,
their femoral bone marrow was flushed out with Eagle's medium, and the marrow was pipetted through needles
of different diameters. The resulting cell suspension was centrifuged at 1000 rpm for 15 min, the residue was
resuspended in 2% Ficoll, and the homogeneous part of the suspension was collected in separate tubes. Homo-
geneity of the suspension was verified cytologically. Electrophoresis of the cells of the resulting bone marrow
suspension was carried out by the method of Griffith et al. [7] in an apparatus for disk electrophoresis (from
Reanal, Hungary). For this purpose the bottom of the tubes was filled with 7.5% polyacrylamide gel on which
layers of 10, 6.25, and 2.5% Ficoll were successively added (Ficoll 400,000, Sweden). The suspension of bone
marrow cells from the intact or experimental mice in equal quantities \( \times 10^4 \), diluted in 2% Ficoll and in 6.8%
sucrose, was poured above, and finally a layer of phosphate buffer, pH 7.2, was carefully poured on the top. In
all cases the Ficoll was mixed with appropriate amounts of sucrose [7]. Suspensions from experimental and
control animals were subjected to electrophoresis simultaneously. The conditions of fractionation were: cur-
rent 1 mA per tube, voltage 8 V applied to the tube, duration of fractionation 1 h. During electrophoresis the
suspension of bone marrow cells of the control and experimental mice was separated into a number of fractions
(Fig. 2). Fractions 2 and 4, located at the edges of the Ficoll gradient, were most clearly revealed. Above
these fractions, and after them, other cells also were found, but their boundaries were indistinct. After the
end of electrophoresis, identical fractions, the boundaries for collection of which are indicated in Fig. 2, were
pooled from different tubes and washed with Eagle's medium to remove Ficoll, after which the absolute num-
ber of cells in each fraction was determined. The percentage of viable cells in the fractions after staining with
trypan blue varied between 80 and 92. Parallel series of films were prepared from each fraction and stained
by the Romanovsky–Giemsa method, after which 500 bone marrow cells were counted and the "myelogram"
analyzed. The results were subjected to statistical analysis.