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A DIFFERENTIATION FACTOR IS PRESENT IN BONE MARROW AND BLOOD SERUM OF NORMAL INDIVIDUALS AND PATIENTS WITH ACUTE LEUKEMIAS

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Interest is increasing in the subject of factors activating cell differentiation both during early embryonic development and during differentiation of hematopoietic stem cells in the adult [2, 4, 5, 7]. Differentiation factors (DF) help to determine the fat of cells in the early stages of embryogenesis, but in adult animals they are essential compounds of the extracellular environment, which plays a leading role in the differentiation of stem and semistem cells [4, 5]. Whereas the role of DF in embryogenesis has been studied in fair detail, the role of DF in adult organisms has not yet been adequately investigated. Studies of the forced differentiation of malignant hematopoietic cells provide evidence of the influence of DF on these processes [1]. One of the most widely distributed DF is the factor which activates differentiation of mesodermal cell types: notochord, muscles, blood cells, mesenchyme, and mesothelium. It has been called mesodermalizing factor (MF) because of its ability to induce mesodermal derivatives in early development [10]. In the adult, it has been called systemic connective-tissue morphogen (SCTM), because of the direction of its action and its localization [4]. SCTM in adult animals is located in the extracellular matrices, bone, cartilage, blood serum [3, 8], and bone marrow [11, 12], and is synthesized by lymphocytes. It has been shown in experimental animals (rats, guinea pigs) that DF with mesodermalizing activity is not synthesized in the bone marrow in leukemia [11]. However, these observations have not been confirmed in man during the development of acute leukemias.

The aims of this investigation were: 1) to detect SCTM in bone marrow of healthy blood donors and to compare its concentration with that in the bone marrow of patients with acute

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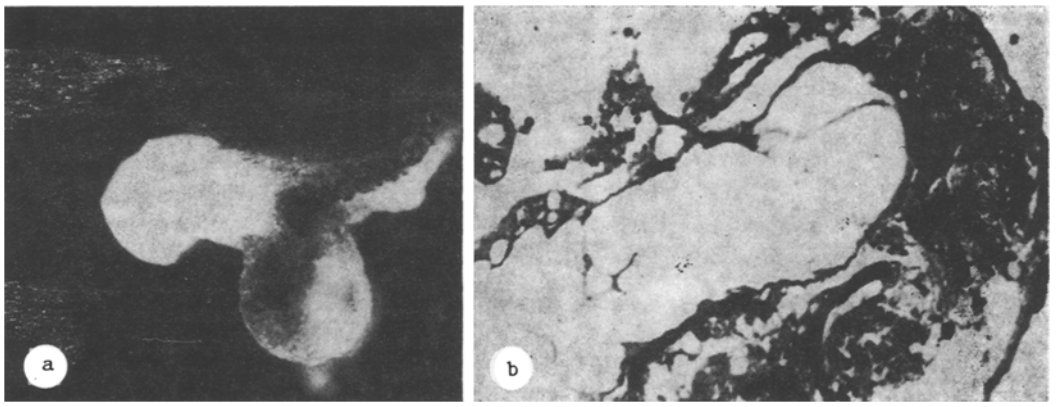

Fig. 1. Development of early embryonic cells. a) Example of trunk-tail structure developing from amphibian EEC under the influence of healthy human bone marrow (400 ×); b) transverse section through trunk-tail structure: notochord and cross-striated muscles can be seen (8000 ×).

TABLE 1. Induction Activity of Bone Marrow from Patients with Different Forms of Acute Leukemia

No. of patient	Recurrence	Number of EEC cultures	Mesodermalizing activity of bone marrow absent or weak
3	ALL	29	0
6 (recurrence)	ALL	16	0
7	ALL	16	0
11	AML	16	0
12	AML	14	0
13 (recurrence)	AML	16	0
15 (recurrence)	AML	8	0
16	AML	8	0
20	AMML	16	0
21	AMML	14	0
22	AMML	8	0
1	ALL	19	+
2	ALL	10	+
8	ALL	8	+
9 remission	AML	16	+
14	AML	10	+
17	AML	7	+
18	AMML	18	+
19	AMML	16	+

Legend. 0) Absence of induction, +) weak mesodermalizing induction.

leukemias; 2) to investigate correlation between its concentrations in the peripheral blood and bone marrow of healthy blood donors and patients with acute leukemias.

EXPERIMENTAL METHOD

Samples of bone marrow obtained by puncture and blood serum from six patients with acute lymphoblastic leukemia (ALL), eight patients with acute monoblastic leukemia (AML), and five patients with acute myelomonoblastic leukemia (AMML), and also bone marrow samples and blood serum from 11 healthy donors with a normal myelogram were investigated. The material was obtained at the No. 1 Hematologic Clinic, Central Research Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, and at the All-Union Oncologic Scientific Center. The DF content was determined by the use of early embryonic cells (EEC) obtained from spur-toed frog (genus Xenopus) embryos at the early gastrula stage. These are totipotent cells and can develop into any of the cell types of the body [9]. Bone marrow puncture material was treated with 70° ethanol. Treating sources of DF with ethanol does not affect their induction activity [11], it eliminates the effect of temperature incompatibility of the reacting tissues, and it ensures better preservation of the EEC, some of which die on contact

with living bone marrow cells. Before the experiment the bone marrow was washed with deionized water containing antibiotics to remove the ethanol. Blood, used to obtain serum, was taken without the addition of sodium citrate or heparin.

The experiments were conducted as follows. Pieces of bone marrow were brought into direct contact with EEC for at least 2 h, i.e., until the end of the period of mesodermal competence of EEC [6]. EEC were added to aliquots of serum (70 µl), in which they were exposed until the time of turning. The EEC were transferred into physiological saline, in which they were cultured for 5 days. The presence of DF and the intensity of their action were determined by the character of the induced structures, on the basis of intravital observations and the results of histological analysis. If DF were present in the test samples, the explants developed into trunk-tail structures (Fig. 1a) and structures with the appearance of vesicles filled with mesodermal cell types. In the absence of DF the EEC developed into atypical epidermis. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Under the influence of the donors' bone marrow several mesodermal cell types appeared: notochord, muscle, blood cells, mesenchyme, mesothelium (Fig. 1b). This proves that human bone marrow contains DF by analogy with the NF found in the bone marrow of rodents [11]. Bone marrow puncture material from healthy donors showed different levels of induction activity: the frequency of appearance of muscles in EEC, for instance, varied from 14 to 81% ($p < 0.05$) and the frequency of appearance of blood cells from 5 to 43%. These variations may be the result of an uneven distribution of DF within the bone marrow and individual variability of DF activity in bone marrow from different donors. Bone marrow puncture material from patients with acute leukemias (Nos. 3, 6, 7, 11, 12, 13, 15, 16, 20, 21, 22) exhibited no induction activity (Table 1): the EEC developed into atypical epidermis, proving that the bone marrow of these patients contained no DF. However, under the influence of bone marrow from patients Nos. 2, 9, 18, and 19, notochords and muscles were induced in between 6 and 14% of cases, whereas under the influence of bone marrow from patients Nos. 1, 8, 14, and 17, mesenchyme, mesothelium, and blood cells were induced in EEC, i.e., the same types of mesodermal cells as, according to the concept of the quantitative mechanism of action of MF, require a low concentration of MF [2, 6]. Patients whose bone marrow exhibited weak mesodermal induction, according to the results of clinical analysis were in a state of remission.

Parallel with discovery of DF in the bone marrow, a series of experiments was carried out to look for DF in the blood serum of healthy blood donors and patients with acute leukemias. This experiment showed that healthy human blood serum induces mesodermal tissues in EEC: muscles, mesenchyme, and mesothelium (10, 30, and 40%). The results show that DF activity in blood serum is significantly weaker than DF activity in healthy human bone marrow ($p < 0.01$). Blood serum from patients Nos. 3, 16, and 22 induced atypical epidermis in EEC, i.e., did not exhibit DF activity; blood serum from patients Nos. 8, 9, 10, and 14 induced differentiation of mesodermal tissues in EEC, but the percentage induction of these cells was lower than normal (Table 1). Analysis of these data shows that DF activity was present in blood serum, just as in bone marrow, if the serum was taken from the patient during a remission.

The results of these experiments were thus as follows: 1) bone marrow puncture material and blood serum from healthy blood donros contain SCTM, the action of which is manifested as induction of mesodermal cell types in EEC; 2) bone marrow and blood serum from patients with acute lymphoblastic, monoblastic, and myelomonoblastic leukemias exhibit no mesodermalizing activity if the patients are in the stage of recurrence of the disease; if, however, the patients are in a remission stage, their bone marrow, like their peripheral blood, exhibit weak mesodermalizing activity; 3) in the same patient correlation is observed between induction activity of the bone marrow and of peripheral blood serum. The suggested method of detection of induction activity can be used as an additional test for the diagnosis of acute leukemia.

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