ONCOLOGY

Spontaneous Death of Bone Marrow and Peripheral Blood Cells during Remission of Acute Lymphoblastic Leukemia in Children

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Programmed antileukemic chemotherapy is associated with prolonged cytotoxic effects on not only tumor cells, but on intact bone marrow and peripheral blood cells as well. The maximum spontaneous cell death is observed immediately after the end of therapy. It decreases with time after therapy, but does not reach the level observed in healthy subjects and does not depend on the type of therapy. The percentage of dead cells in peripheral blood directly correlates with the percentage of dead cells in the bone marrow. The processes of cell death in the peripheral blood and bone marrow are synchronous and parallel.

Key Words: *acute lymphoblastic leukemia; granulocytes; lymphocytes; apoptosis; flow cytometry*

Wide use of intensive programmed drug therapy in children with acute lymphoblastic leukemia (ALL) during the recent decade altered the prognosis for this disease: complete long-term remissions are attained in 70-80% children with ALL [2]. On the other hand, this intensive and highly effective drug therapy affect not only tumor cells, but also many proliferating tissues, first of all hemopoietic cells [3]. Recent studies indicate that the main cytotoxic mechanism of antitumor therapy is induction of apoptosis in target cells [1,2, 4,5]. Delayed myelotoxic effects of drug therapy are still poorly studied. These studies are of particular importance for expert evaluation of the clinical status of children with a history of ALL after therapy. It was previously shown the patients survived ALL have normal leukocyte composition of the blood and serious myelopoiesis disturbances (suppressed activity of the granulocyte-macrophage precursors).

We investigated the delayed changes in spontaneous cell death in the peripheral blood (PB) and bone marrow in children receiving programmed drug therapy for ALL.

MATERIALS AND METHODS

Peripheral blood and bone marrow specimens from 36 children with ALL remissions treated according to protocols ALL BFM-90 (n=22) and ALL MB-91 (n=14) were analyzed. The children were divided into 3 groups depending on the period elapsed after the treatment: 1) up to 1 year after therapy (n=8); 2) 1-2 years (n=11); and 3) more than 2 years (n=17).

Morphological composition of PB was analyzed on an Argos Cobos 5 Diff analyzer, bone marrow specimens were analyzed by light microscopy after hematoxylin and eosin staining.

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Spontaneous apoptosis of bone marrow cells (BMC) and PB lymphocytes and granulocytes was evaluated using a PermoCyte-FO, WBL 1010 kit (Bio-Ergonomics) [7]. To this end, 100 μl whole blood or bone marrow mononuclear fraction isolated in Ficoll density gradient (1.077 g/ml) was centrifugated in 1 ml phosphate saline buffer at 1000 rpm for 5 min. The precipitate was resuspended in 2 ml permeabilizing solution (×1) and promptly fixed in 10-fold fixative. Then the cells were incubated for 30 min at 20°C until complete erythrocyte lysis, washed twice, and stained with propidium iodide (4 μg/ml cell suspension) for 40 min at 37°C. After staining the fluorescence intensity of 10,000 cells was analyzed in a FacScan BD flow cytofluorometer (band II). DNA distribution was analyzed and the percentage of hypodiploid cells in the entire cell population (PB and bone marrow), lymphocytic and granulocytic clusters (for PB) isolated by the photooptic characteristics was estimated.

RESULTS

Apoptosis is an active form of cell death induced by exogenous and endogenous factors and manifesting in deep disorders of cell energy metabolism due to mitochondrial dysfunction. The cell looses genetic material due to partial DNA degradation [1,4]. Apoptosis is a energy-dependent process requiring cell membrane rearrangement and synthesis and activation of some macromolecules. It plays an important role in the maintenance of a constant cell population in organs and the whole organism, regulation of cell populations, elimination of old cells, *etc.* [1-6]. However, the maintenance of cell population homeostasis requires balanced activation of proliferation processes.

TABLE 1. Counts of PB Granulocytes and Lymphocytes (10^3/mm^3) in Groups Receiving Different Therapy (*M*±*m*)

Parameter	Control (*n*=20)	MB-91 (*n*=14)	BFM (*n*=22)
Leukocytes	7.60±0.56	7.40±0.64	7.30±0.32
Granulocytes	4.32±0.24	3.97±0.51	4.12±0.24
Lymphocytes	2.75±0.29	2.09±0.12	2.07±0.11

The counts of PB granulocytes and lymphocytes in patients did not differ from normal, and there were no significant differences between groups of patients with different periods after the therapy (Fig. 1) or patients receiving different schemes of treatment (Table 1). The intensity of spontaneous apoptosis in PB granulocyte and lymphocyte populations after

TABLE 2. Myelogram at Different Terms after Therapy (*M*±*m*)

Parameter	Control (*n*=65)	Period after therapy, years		
		<1	1-2	>2
Granulocytes, %				
proliferating (P)	18.9±0.7	14.3±2.1	14.6±1.9	13.2±1.8
nonproliferating (N)	38.3±0.9	38.1±7.2	38.8±3.8	38.1±5.4
P/N	0.49±0.07	0.48±0.16	0.51±0.11	0.35±0.03

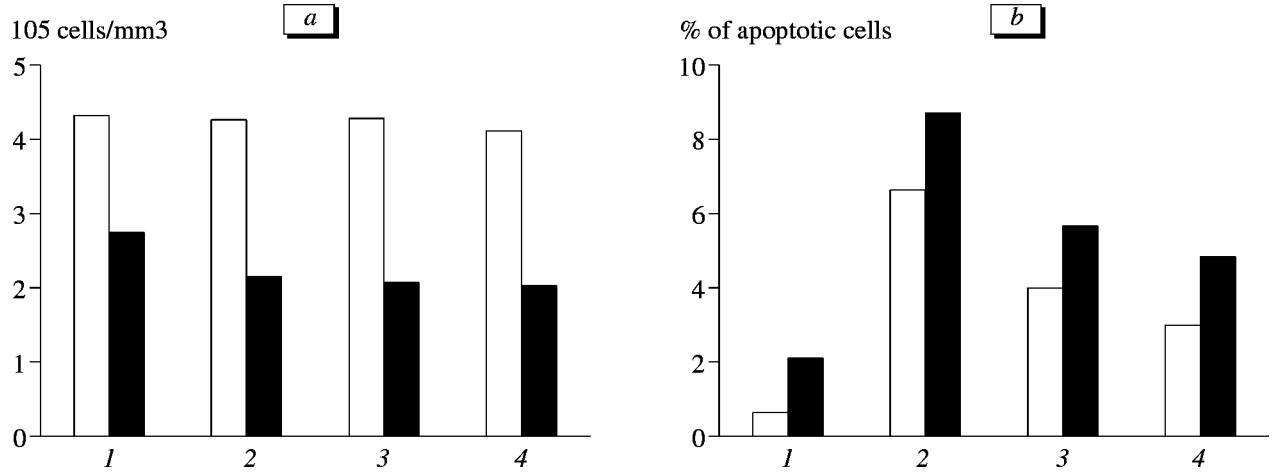

Fig. 1. Counts (*a*) of granulocytes (light bars) and lymphocytes (dark bars) and level of apoptosis (*b*) for granulocytes and lymphocytes (light and dark bars, respectively) in patients with acute lymphoblastic leukemia at different terms after therapy. *1*) control; *2*) <1 year; *3*) 1-2 years; *4*) >2 years.

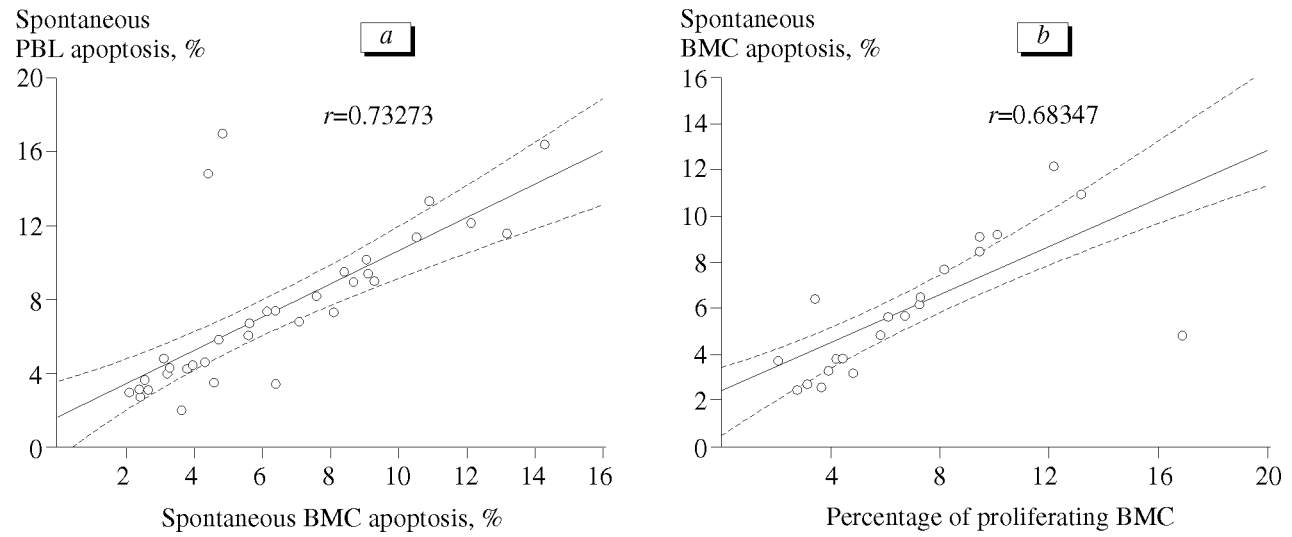

Fig. 2. Correlation between the level of spontaneous apoptosis of bone marrow cells (BMC) and peripheral blood leukocytes (PBL) (*a*) and between percentage of proliferating BMC and level of spontaneous PBL apoptosis (*b*).

chemotherapy considerably increased (Fig. 1). This parameter decreased with time, but did not returned to the control level.

The content of precursor cells (main component of hemopoiesis reproduction), percentage of proliferating and nonproliferating granulocytes, and level of spontaneous BMC apoptosis were studied in bone marrow puncture biopsy specimens. Spontaneous BMC apoptosis was estimated for the total population of bone marrow nuclears except mature neutrophils (mononuclear fraction of the bone marrow). The data indicate that the level of spontaneous apoptosis decreased significantly in patients treated more than 2 years before (5.79±0.95% *vs.* 7.55±1.49 and 8.55±0.90%, $p<0.05$, in children treated 1 years and 1-2 years before the present study, respectively). The intensity of apoptosis of BMC and PB cells were in positive correlation (Fig. 2, *a*). This suggests that stimulation of apoptosis is a universal delayed effect of chemotherapy manifesting similarly in all components of hemopoiesis. However, the positive correlation between the percentage of proliferating cells and the level of spontaneous BMC apoptosis in groups 1 and 2 and increased percentage of proliferating BMC in these groups (Table 2) suggest that enhancement of spontaneous apoptosis of myeloid cells during the first 2 years after chemotherapy is compensated by increased proliferative pool in the bone marrow (Fig. 2, *b*).

Hence, our findings indicate prolonged delayed proapoptotic effect of antileukemic drug therapy on all components of hemopoiesis. The intensity of spontaneous apoptosis decreases with time after therapy. Despite increased level of spontaneous apoptosis, leukocyte counts in PB of patients at all terms after therapy were maintained at a normal level, which was presumably due to enhanced proliferation of BMC. We revealed no differences in the intensity of cell death processes in the patients treated according to BFM-91 and MB-91 protocols.

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