INDUCED DIFFERENTIATION OF LEUKEMIC CELL SUBPOPULATIONS IN THE BLOOD OF PATIENTS WITH CHRONIC B-CELL LYMPHATIC LEUKEMIA

E. N. Zlobina and A. Yu. Baryshnikov

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The immunological heterogeneity of leukemic cells in patients with chronic B-cell lymphatic leukemia (B-CLL) has now been conclusively demonstrated [1-4]. The causes of this heterogeneity have not been fully studied. Since the marker of the early stage of differentiation of normal B lymphocytes, namely the receptor for mouse erythrocytes (M-receptor), is expressed on only half of all leukemic cells [5, 6], it can be tentatively suggested that cells of patients with B-CLL are at different levels of differentiation.

The aim of this investigation was to compare the immunologic phenotype of cells forming and not forming M-rosettes, and also the possibility of induction of differentiation in them under the influence of 12,0-tetradecanoylphorbol-13-acetate (TPA).

EXPERIMENTAL METHOD

Peripheral blood lymphocytes from patients with B-CLL were isolated on a Ficoll-Verografin density gradient (d = 1.076). T cells were removed by the method of E-rosette formation. The M-rosette formation reaction was carried out with the remaining cells and cells forming M-rosettes (M^+) were separated from those not forming M-rosettes (M^-) on a Ficoll-Verografin density gradient (d = 1.076). Expression of Ia-like antigens (AG) was determined on unfractionated M^+- and M^--lymphocytes in the indirect immunofluorescence test (IFT). Expression of surface and cytoplasmic immunoglobulins (sIG and cIG, respectively) by the cells was determined in the direct IFT with the aid of polyvalent and nonspecific antisera to human IG (from the N.F. Gameleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR). Unfractionated lymphocytes and also M^+- and M^--cells were cultured separated in medium RPMI-1640 with the addition of 20% embryonic calf serum in a concentration of (1-1.2) $\times 10^6$ cells/ml medium in 24-well plates (Flow Laboratories, England). TPA in a dose of 20 ng/ml was added to each experimental well. After every 2, 5, 6, and 7 days expression of immunologic markers (Ia-like AG, sIG, and cIG) was determined on these cells. Control data were obtained by testing the cells of these same patients, cultured without the addition of TPA, at the same times as after addition of TPA.

EXPERIMENTAL RESULTS

Two different subpopulations of cells were found in the peripheral blood of patients with B-CLL: M^+) carrying M receptors, and M^-) not carrying M receptors. Data for the subpopulations of M^+- and M^--cells are given in Table 1. Compared with unfractionated lymphocytes, the subpopulation of M^+-lymphocytes contained a significantly lower percentage of cells expressing sIG and cIG. The subpopulation of M^--cells contained statistically significantly fewer cells than the unfractionated lymphocytes which expressed Ia-like AG. M^+- and M^--cells differed significantly from each other. On the one hand, significantly more M^+-cells than M^-expressed Ia-like AG, and on the other hand, significantly more M^--cells contained cIG, whereas M^+-cells contained virtually no cIG (Table 1).

In 9 of 18 cases the immunologic phenotype of the blood cells of patients with B-CLL, incubated with TPA, was changed (Fig. 1). For example, after 2 days the number of cells carrying M receptors and sIG was reduced, and cells containing cIG appeared. After 5 days

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TABLE 1. Immunologic Markers of Peripheral Blood Lymphocytes of Patients with B-CLL, Unfractionated and Fractionated by M-Rosette Formation

Material tested	Ia-like antigen			S/G			C/G		
	I	II	III	I	II	III	I	II	III
1, Unfractionated lymphocytes	52/52	100	58,1±2,8	37/52	71,1	27,7±4,3	21/52	40,4	46,3±7,1
2. Fractionated lymphocytes carrying M receptors (M+)	49/49	100	63,2±3,3	35/49	71,4	17,5±2,7	8/46	17,4	14,7±5,6
3. Fractionated lymphocytes not carrying M receptors (M^-)	49/49	100	49,6±2,9	21/49	42,8	22,4±5,3	4/26	52,2	34,5±5,3
P_{1-2}		>0,05			<0,05			<0,05	
P_{1-3}		<0,05			>0,05			>0,05	
P_{2-3}		<0,01			>0,05			<0,05	

Legend. I) Numerator — number of positive cases, denominator — total number tested; II) frequency of expression of marker (in percent); III) percentage of positive cells (M ± m).

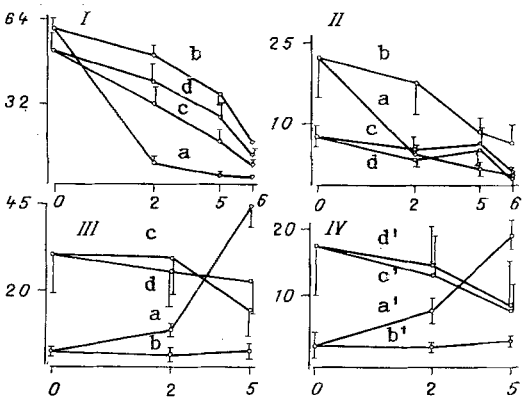

Fig. 1. Time course of expression of immunologic markers on leukemic blood cells from patients with B-CLL, cultured with TPA. Abscissa, period of culture (in days); ordinate, percentage of unfractionated lymphocytes with M receptors (I), sIG (II), and cIG (III) and percentage of fractionated M^+- and M^--lymphocytes with cIG (IV). a) "Responding" cells cultured with TPA (experiment); b) "responding" cells cultured without TPA (control); c) "nonresponding" cells cultured with TPA (experiment); d) "nonresponding" cells cultured wihtout TPA (control); a') M^+-cells cultured with TPA (experiment); b') M^+-cells cultured without TPA (control); c') M^--cells cultured with TPA (experiment); d') M^--cells cultured without TPA (control).

virtually no sIG and M receptors were present on the cells. The number of cIG-containing cells rose to 45.4 ± 6.1%. The time course of expression of Ia-like AG was virtually indeterminable. Initially the phenotype of these cells which "responded" to TPA was characterized by expression of Ia-like AG, M receptors, and sIG and by the absence of cIG. In the remaining 9 cases the immunologic phenotype of cells "not responding" to TPA in patients with B-CLL had no significant changes with time (Fig. 1). Before addition of TPA to the culture the "nonresponding" cells expressed Ia-like AG and M receptors, but virtually did not carry sIG; 30.7 ± 6.1% of "nonresponding" cells initially contained cIG.

Under the influence of TPA changes also took place in the immunologic phenotype of the M^+-cells. For instance, after 2 days the number of M receptors and sIG fell sharply. Although the M^+-cells did not contain cIG before addition of TPA, two days after the addition

of TPA a small percentage of cIG-containing cells appeared (Fig. 1). After 5 days these cells did not carry M receptors and sIG, but 19.2 ± 1.9% of the cells contained cIG. Initially the M^--cells did not express M receptors or sIG, but 17.4 ± 9.5% of these cells contained cIG. The action of TPA did not lead to any significant changes in the immunologic phenotype of the M^--cells (Fig. 1).

Thus cells belonging to M^+- and M^--subpopulations coexist in different proportions in the peripheral blood of patients with B-CLL, and the M^+-lymphocytes express significantly more Ia-like AG than the M^--cells, they contain hardly any cIG, and they are evidently less mature cells. The M^--lymphocytes express fewer Ia-like AG but contain far more cIG than the M^+-lymphocytes, and they are evidently more mature cells. Lymphocytes of patients with B-CLL respond heterogeneously to TPA. The less mature cells, on which sIG are expressed, differentiate but the more mature, cIG-containing cells do not differentiate. Differentiation is accompanied by loss of the features distinguishing the less mature B-cells, namely M receptors and sIG, and by the acquisition of features of the more mature cells, namely accumulation of cIG. Subpopulations of leukemic cells in each patients differ in their responce to TPA. Under the influence of TPA the less mature M^+-cells differentiate, and under these circumstances the number of cIG-containing cells increases. The more mature cIG-containing M^--cells do not differentiate under the influence of TPA.

It has been suggested that cells of patients with B-CLL preserve some of their ability to differentiate. However, this ability depends on the immunologic maturity of the leukemic cells. Differentiation is induced in the less mature, sIG-carrying cells. Since at least two subpopulations of M^+- and M^--cells, differing in their degree of maturity, coexist in the blood of patients with B-CLL, only one of them, the subpopulation of less mature M^+-cells, preserves its ability to undergo further differentiation. Further maturation of leukemic B-lymphocytes may perhaps take place on account of differentiation of immature M^+-cells in the course of the disease, and on the whole this gives the pictures of heterogeneity of the immunologic phenotype in B-CLL.

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