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CHROMOSOME EXAMINATIONS ON SIX-HOUR CULTURES OF UNSTIMULATED PERIPHERAL BLOOD FROM SOME PATIENTS WITH CHILDHOOD LEUKEMIA

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Six-hour cultures of unstimulated peripheral blood cells from patients with various types of childhood leukemias were examined for chromosome karyotypes. It was found that this method was suitable for the detection of characteristic chromosomal abnormalities in two cases of acute nonlymphoblastic leukemias (ANLL; FAB types M3 and M6) and in a case of chronic myelogenous leukemia (CML), but not in acute lymphoblastic leukemias (ALL). The results suggest the usefulness of this simple method (possibly in combination with the thymidine boost technique of Yunis) in the cytogenetic diagnosis of some types of leukemias.

INTRODUCTION

With the present techniques of flow cytometry, it is possible to investigate the frequency distributions of normal and leukemic cells in the different phases of the cell cycle ($G_{0/1}$, S, G2+M) /1, 4, 5, 7, 10, 11, 19/. From these studies, it has turned out that the number of cells in phase G_2+M of the cell cycle is below 1% in peripheral blood samples from normal healthy donors /1, 11/. In contrast with this finding, a higher percentage (2.5-84%) of cells in phase G2+M was found in the peripheral blood of patients with various types of leukemias /1, 4, 5, 7, 10, 19/. The duration of phase G_2 of the normal cell cycle is 4 hours in human leukocytes /6/, and leukemic cells have a longer generation time /6, 13/ than normal cells.

On the basis of these results we have investigated the usefulness of a 6 hour culture method for cytogenetic examinations of childhood leukemias. It was found that this

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method is capable of demonstrating the chromosome content in some types of leukemias, in contrast with acute lymphoblastic leukemias (ALL), in which no dividing cells were detected.

PATIENTS AND METHODS

Patients

Case 1: P.H., a 9-year-old boy with erythroleukemia (ANLL; FAB type M6). The WBC was 16.0 x 10^9/l, with 5% neutrophils, 7% myelocytes, 3% metamyelocytes, 3% monocytes, 67% lymphocytes, 12% lymphoblasts and 3% erythroblasts; the platelet count was 25.0 x 10^9/l. Chromosome examinations were made in the course of therapy with alexan, lanvis and methipred. This case will be published as a case report in "Haematologia ".

Case 2: I.N., a 9-year-old boy with Ph^1-positive CML. (Chromosome examinations were earlier made on bone marrow cells and on a 24-h unstimulated whole blood culture.)(The WBC was 14.0 X 10^9/l, with 39% atypical blasts, 40% lymphocytes, 6% myeloblasts, 11% myelocytes, 2% eosinophils and 2% monocytes. Chromosome examinations were made in the course of supportive treatment.

Case 3: A.P., a 2-year-old girl with acute promyelocytic leukemia (ANLL; FAB type M3). The WBC was 28.0 x 10^9/l, with 43% neutrophils, 3% promyelocytes, 4% metamyelocytes, 1% myelocytes, 1% basophils, 28% lymphocytes, 9% blasts, 11% monocytes; the platelet count was 30.0 x 10^9/l. Chromosome examinations were made before the start of therapy.

Case 4: H.C., a 4-year-old girl with ALL (L1). The WBC was 40.0 x 10^9/l, with 2% neutrophils, 1% bands, 77% lymphocytes and 20% lymphoblasts; the platelet count was 115.0 x 10^9/l. Chromosome examinations were made before the start of therapy.

Case 5: A.S., a 5-year-old girl with ALL (L1). The WBC was 5.9 x 10^9/l, with 50% neutrophils, 9% bands, 32% lymphocytes, 2% lymphoblasts and 7% atypical blasts. Chromosome examinations were made before the beginning of therapy.

Case 6: A.S., a 7-year-old boy with ALL (L1). The WBC was 1.2 x 10^9/l, with 9% neutrophils, 3% bands, 77% lymphocytes, 10% lymphoblasts and 1% eosinophils. Chromosomes were investigated before the start of therapy.

Subjects 7-9: Two healthy girls and one healthy boy served as controls; they were age-matched with the cases (the were 2, 5 and 9 years old).

Cytogenetic studies

Six-hour cultures of unstimulated peripheral blood cells from patients were examined for chromosome karyotypes by using a standard technique (3, 23). Briefly, 2 ml of peripheral blood was incubated in 10 ml of TC 199 (Difco) medium at 37°C for 6 hours. Vinblastin (0.5 µg/ml medium) was added to block the mitoses 1 or 1.5 hours before harvesting. The hypotonization was made with 0.075 M KCl for 25 minutes, and the cells were

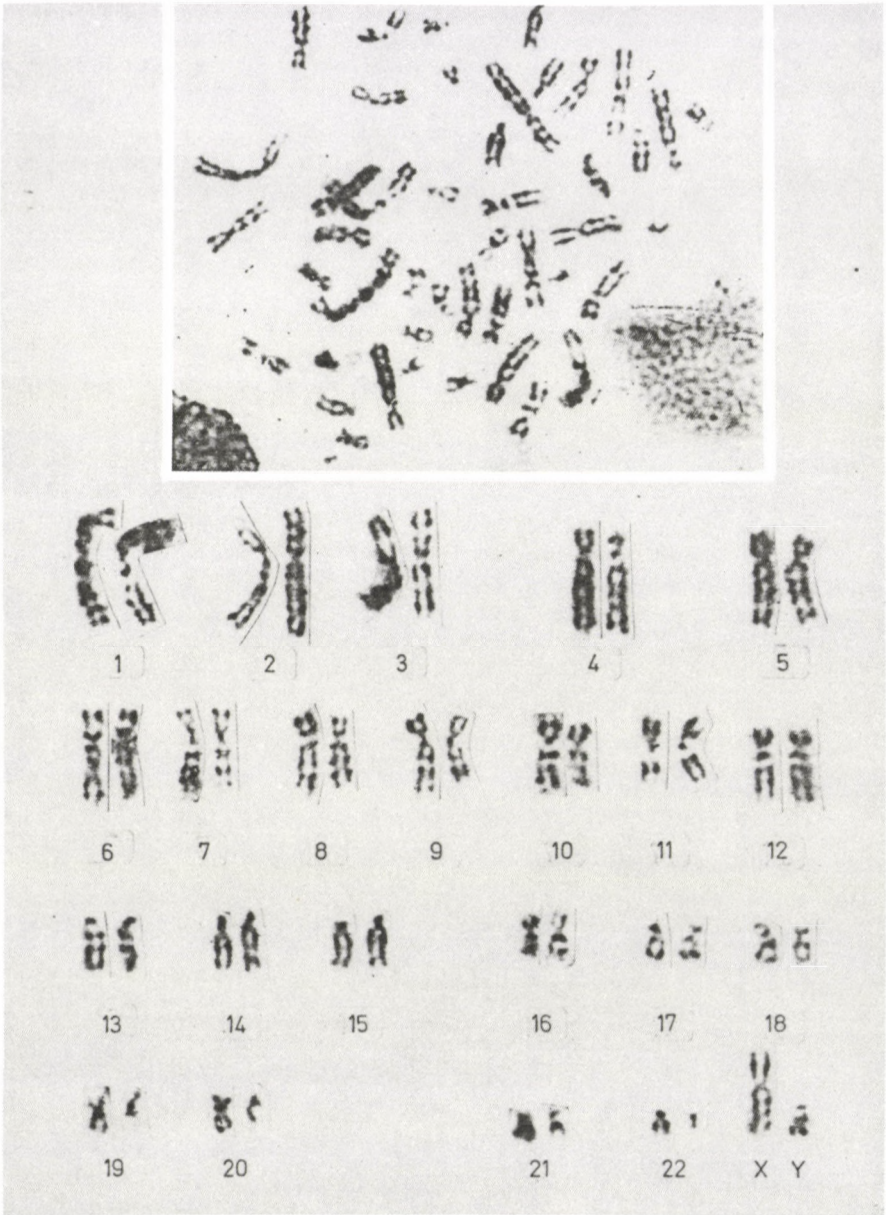

Fig. 1. The karyotype shows the characteristic aberration t (9 ; 22) (q34; q11) for CML (patient 2).

then fixed four times with methanol:acetic acid 3:1. The fixed cells were transferred to glass slides; treated with trypsin /22/, stained with Giemsa, and examined under a microscope at a magnification of 1600 X. Karyotypes were analysed according to ISCN /12/.

In one case (patient 3), the thymidine boost technique /24/ was used (the cells were exposed to 10^{-5} M/l thymidine during the culture period) with a 30-minute Vinblastin exposure.

RESULTS

Table I shows the chromosome examination data. It can be seen that from the unstimulated blood of patients No 1-3, 35-57 metaphases could be examined under the microscope, in contrast with patients No 4, in the blood cultures of whom only 2 metaphases were found. Two-seven mitoses were detected in blood cultures from the healthy controls.

Table II shows the karyotyping data. It can be seen that in the unstimulated blood cultures from the healthy controls, only a few normal metaphases were found, in contrast with patients No 1-3, in whose blood cultures the vast majority of the mitoses showed numerical or structural aberrations (93.3%). From blood cultures from patients No 4-6, we were unable to prepare any karyotypes.

DISCUSSION

On the basis of the results presented here, it seems that the 6-hour culture method was suitable for the detection of characteristic chromosome aberrations relating to various types of leukemias in the unstimulated peripheral blood from the patients. This was proved by /1/ the presence of Philadelphia chromosome (Ph^1) and the characteristic translocation t (9; 22) (q34; q11) for CML /17, 20/ in the blood culture from patient No 2; /2/ the characteristic translocation t (15; 17) (q22; q11) for promyelocytic leukemia /21/ in the blood culture from patient No 3; and /3/ del (5q) and -7 characteristic

TABLE I

Chromosome examination data

Patient			Type of leukemia	Number of metaphases examined under the microscope	Number of karyotypes examined
No	Sex	Age (years)			
1	M	9	ANLL; M6	40	19
2	M	9	CML	35	10
3	F	2	ANLL; M3	57	9
4	F	4	ALL	2	0
5	F	5	ALL	0	0
6	M	7	ALL	0	0
7	F	20	healthy control	2	2
8	F	37	healthy control	3	2
9	M	37	healthy control	7	4

TABLE II

Karyotype analysis data

Patient No	Type of Leukemia	Type of aberration	Number of cells with aberration
1	ANLL; M6	46,XY	2+
		46,XY,del/5//q21-q23/	4
		46,XY,-7,+mar,del/5//q21-q23/	2
		47,XY,+mar,del/5//q21-q23/	3
2	CML	46,XY,t/9;22//q34;q11/	10
3	ANLL; M3	45,XX,-22,t/15;17//q22;q11/	1
		46,XX,t/15;17//q22;q11/	8
7	healthy control	46,XX	without aberration 2
8	healthy control	46,XX	2
9	healthy control	46,XY	4

+ This case will be published as a case report in "Haematologia", with complete karyotype analysis.

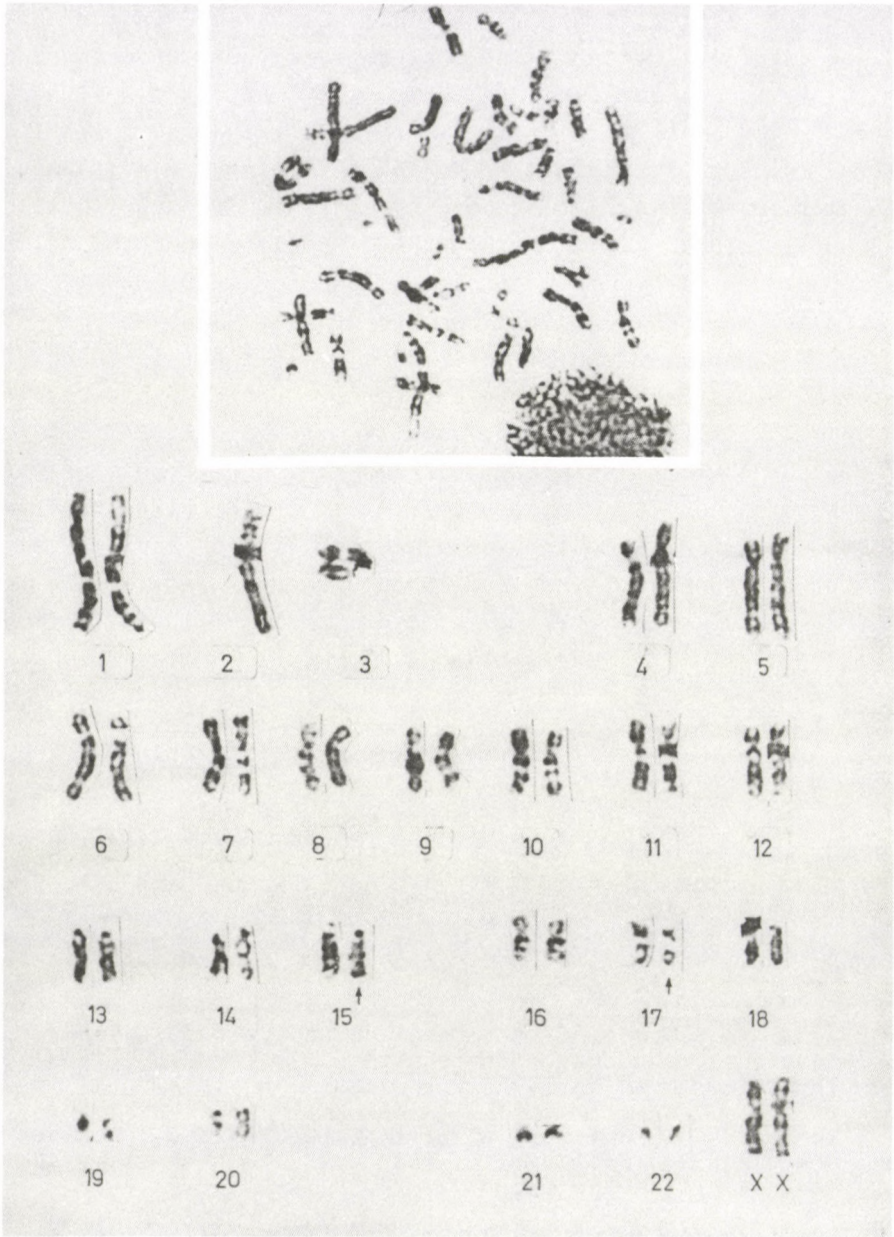

Fig. 2. The karyotype shows the characteristic aberration t (15; 17) (q22; q11) for promyelocytic leukemia (ANLL; M3) (patient 3).

chromosomal abnomalities of EL /2, 8, 16, 21/ in the blood culture from patient No 1. No analysable mitoses were found in the blood cultures from patients with ALL, and only a few normal metaphases were present in the blood cultures from healthy subjects.In patient No 3, the thymidine boost technique /24/ too was used, to prepare chromosomes from unstimulated peripheral blood. This method gave chromosomes in better quality than the simple method; thus, both methods can be used in parallel in the investigations. In contrast with our method, 24 or 48-hour cultures of unstimulated peripheral blood from patients with leukemia have previously been investigated for conventional cytogenetic analysis /9, 14, 15, 18, 24/. It can be stated that this simple 6-hour culture of unstimulated peripheral blood from patients with CML or ANLL (FAB types M3 and M6) was suitable for the detection of the characteristic chromosomal abnormalities in these diseases, and this method can be useful in the cytogenetic diagnosis of these types of leukemias, but not in cases of ALL.

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