Cytogenetic Analysis of Different Cellular Populations in Chronic Myelomonocytic Leukemia

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ABSTRACT: *Karyotypes of different cellular populations made after separation of bone marrow cells on a gradient of Percoll were evaluated in seven patients affected by chronic myelomonocytic leukemia diagnosed according to FAB criteria. Megakaryocytes, monocytic cells, and granulocytic and erythroid precursors were preferentially collected after centrifugation between density layers of 1045–1050 mg/ml, 1050–1060 mg/ml, and 1065–1070 mg/ml, respectively. The enriched cell fractions were cultured separately and submitted to cytogenetic investigation after short-term culture. Some chromosome aberrations (5q−, +8) were observed in all cellular fractions in three patients, thus providing cytogenetic evidence of the involvement of a common progenitor stem cell in this myelodysplastic disorder. On the other hand, chromosome abnormalities such as del(3)(q21) and del(11)(q23) appeared to be confined to the megakaryocytic and the monocytic fractions, respectively, in two patients. It is conceivable that lineage-restricted aberrations may develop as a consequence of a multistep clonal evolution and may show a close relationship with the hemopoietic differentiative processes.*

INTRODUCTION

Chronic myelomonocytic leukemia (CMMol) is a myeloproliferative disorder recently included by the FAB Cooperative Group among the myelodysplastic syndromes [1]. This classification, while introducing a sharper definition of the disease, has provided the basis for studies leading to a better delineation of its clinical and biologic aspects [2, 3]. The cytogenetic features of CMMoL have recently been evaluated in studies carried out on a large series of patients, confirming the observation of previous reports [3, 4] of the occurrence of nonrandom chromosome abnormalities in about 30% of the cases. Most of these aberrations are not specific for this disease and appear to be shared by other myeloid malignancies [5, 6].

Since CMMoL is considered a disorder of a pluripotent progenitor stem cell [7], the involvement of multiple hemopoietic lineages is a common feature in most of the patients. Thus the cytogenetic analysis of bone marrow cells separated on a density gradient [8] in order to obtain relatively pure cell populations may repre-

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Table 1 Laboratory features in seven patients with CMMoL.

Patient sex/age	Hb (g/dl)	WBC (×10⁹/L)	%Mono-l (PB)	Platelets (×10⁹/L)	Serum lysozyme	M/E	BM aspiraton		Unfractionated BM karyotypes (24–48 h)	No. of cells
							%mono-l	Cytologic features		
1. F/79 yr	9.5	25	22	200	30 mg/L	8/1	25	Megaloblastoid change of erythroid precursors—5% atypical monocytes	NN 45,XX,−16 46,XX,del(11)(q23)	11 3 1
2. M/59 yr	9.7	21.2	10	650	29 mg/L	6/1	28	Micromegakaryocytes, numerous sideroblasts	NN 46,XY,del(3)(q21) 47,XY,+21	12 2 1
3. M/64 yr	9.9	6.8	41	180	16 mg/L	7/1	16	Nuclear erythroid aberrations—occasional ringed sideroblasts	NN 46,XY,del(5)(q31) 47,XY,+20,del(5)(q31) 44,XY,−19,−22,del(5)(q31)	8 4 2 1
4. M/68 yr	12.1	5.1	33	290	nd	5/1	13	Hypogranular neutrophil leukocytes	nd	
5. F/61 yr	9.9	12.5	15	158	8 mg/L	6/1	18	Hypogranular and hyposegmented neutrophil leukocytes	NN	9
6. F/54 yr	10.4	8.4	20	85	19 mg/L	8/1	22	Hypolobular neutrophils—nuclear dyserythropoiesis	46,XX,+8, −18 47,XX,+8 NN	4 3 5
7. F/77 yr	11.1	14.5	10	163	9 mg/L	5/1	9	Multinuclear erythroblasts	NN	11

Abbreviations: Mono-l, cells of the monocytic lineage; PB, peripheral blood; BM, bone marrow; Serum lysozyme, nv = 5–9 mg/L; M/E, myelo-erythroid ratio; nd, not done; NN, normal karyotype.

sent an interesting tool for the assessment of the distribution of the chromosome abnormalities and the detection of lineage-restricted aberrations. The results of a cytogenetic study performed on bone marrow cells after separation on a Percoll density gradient are presented and discussed in this report.

PATIENTS AND METHODS

The patients were admitted to our institution between January 1984 and December 1986, and diagnosis was made in accordance with the FAB criteria [1]. Bone marrow aspiration smears were stained both with the May Grunwald–Giemsa method and with cytochemical reactions [Prussian Blue, Sudan Black B (SBB), naphthol AS-D chloroacetate esterase (NASDCAE), and alpha-naphthyl acetate esterase (ANAE) with and without fluoride inhibition].

Separation of Bone Marrow Cells

Mononuclear cells (30×10^6) were layered on top of a Percoll gradient in sterile polypropylene tubes and centrifuged at 600 g for 30 minutes. The densities used were 1070 mg/ml, 1065 mg/ml, 1060 mg/ml, 1055 mg/ml, 1050 mg/ml, and 1045 mg/

Figure 1 Cellular populations at different Percoll densities are shown. a. 1060–1050 mg/ml fraction. The cytospin preparation was stained with combined reactions for ANAE and NASDCAE. A scattered granular positivity for the nonspecific esterase is present in most of the elements. A granulocytic precursor positively stained for NASDCAE is arrowed. b. Cells collected at 1050–1045 mg/ml density stained with anti-factor VIII-related antigen monoclonal antibody coupled with the immunoalkaline phosphatase technique are shown.
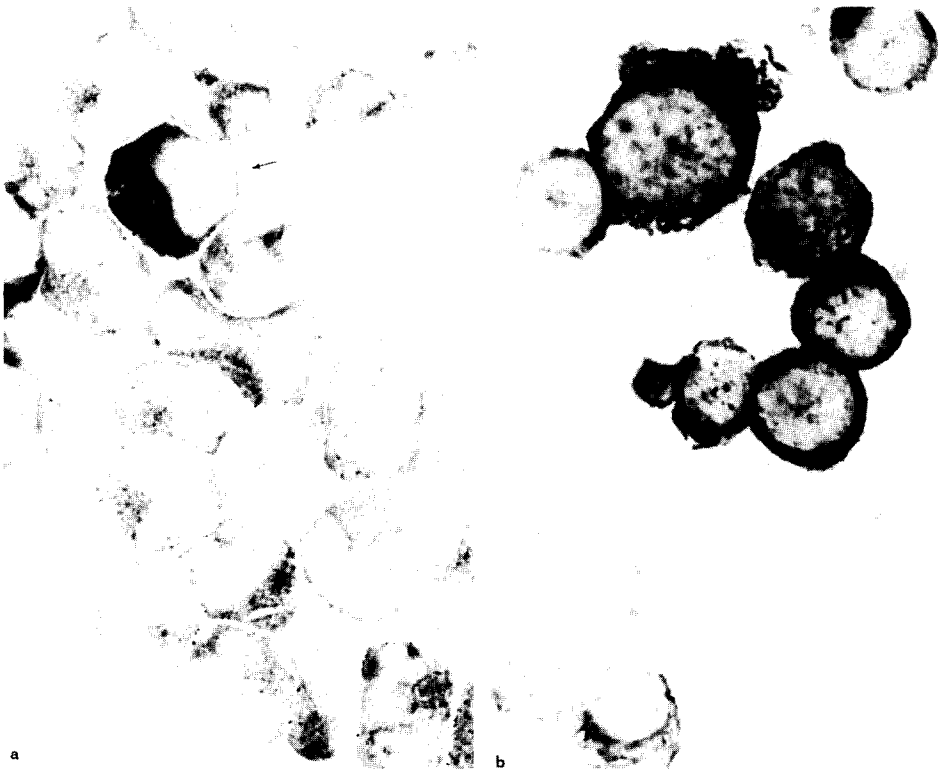
ml from the bottom to the top, and the volume of each layer was 1.2 ml. Fractionated cellular populations were collected at the interfaces of the different densities, washed twice in TC Medium 199 (GIBCO), and cultured in RPMI-1640 medium (GIBCO) supplemented with 15% fetal calf serum and antibiotics at 37°C in 5% CO_2 humidified atmosphere. Cytospin preparations obtained from each culture were stained by means of cytochemical and cytoimmunologic procedures [periodic acid-Schiff, NASDCAE, ANAE (+NaF), anti-factor VIII related antigen monoclonal antibody coupled with immunoalkaline phosphatase technique] for a cytologic characterization.

Cytogenetic Investigation

Each cell fraction was processed for chromosome analysis according to conventional procedures after 6–72 hours' culture incubation. Air-dried slides were submitted to ASG and trypsin banding methods [9, 10]. As many metaphases as possible of each preparation were analyzed and karyotyped according to the ISCN nomenclature [11].

RESULTS

The data concerning the clinical and hematologic appearance at presentation confirmed the existence of distinct characteristics in this myelodysplastic syndrome [2]. Bone marrow was hypercellular in all the patients, with a marked increase of granulomonocytic precursors. Five patients presented important dyserythropoietic features; dysgranulopoiesis (three cases) and marked dysmegakaryocytopoiesis (one case) were also observed. Detailed hematologic data and unfractionated bone marrow karyotypes are given in Table 1.

The separation of bone marrow cells was successful in all patients, and the en-

Table 2   Distribution patterns of various cell populations in seven patients with CMMoL assessed by cytochemical and cytoimmunologic techniques

| Patient | Prevailing cytologic type at Percoll densities | | |
	1070–1065 mg/ml	1060–1050 mg/ml	<1050 mg/ml
1.	Ebls: 21%	Mono-l: 48%	Mono-l + Mybls: 70%
	PM-M: 72%	Mybls: 33%	Mk-l: 22%
2.	Ebls: 27%	Mono-l: 43%	Mono-l + Mybls: 54%
	PM-M: 68%	Mybls: 41%	Mk-l: 38%
3.	Ebls: 48%	Mono-l: 18%	Mono-l + Mybls: 81%
	PM-M: 45%	Mybls: 71%	Mk-l: 11%
4.	Ebls: 44%	Mono-l: 56%	Mono-l + Mybls: 86%
	PM-M: 50%	Mybls: 40%	Mk-l: 8%
5.	Ebls: 28%	Mono-l: 71%	Mono-l + Mybls: 89%
	PM-M: 61%	Mybls: 26%	
6.	Ebls: 44%	Mono-l: 22%	Mono-l + Mybls: 78%
	PM-M: 54%	Mybls: 76%	Mk-l: 10%
7.	Ebls: 31%	Mono-l: 40%	Mono-l + Mybls: 73%
	PM-M: 63%	Mybls: 47%	Mk-l: 19%

Differential on cytospin preparation slides (200 cells observed).

Abbreviations: Ebls, Erythroblasts; PM-M, promyelocytes and myelocytes; Mono-l, cells of the monocytic lineage; Mybls, myeloblasts; Mk-l, cells of the megakaryocytic lineage.

riched cellular fractions collected between the 1970–1065, 1060–1055–1050, and 1050–1045 mg/ml densities were suitable for cytogenetic investigation. As revealed by the staining procedures, these fractions were constituted by erythroblasts and granulocytic precursors, by myeloblasts and monocytic cells, by megakaryocytes, and by both myeloid and monocytic immature cells, respectively, (Fig. 1). The distribution pattern of various cell populations is given in Table 2.

The cytogenetic investigations were performed after a 6-hour incubation from the erythroblast enriched fraction, after 24–48 hours from the monocytic fraction, and after 24–72 hours from the megakaryocyte-enriched cellular population. Results were reported in Table 3. All the cultured cellular fractions exhibited a trisomy of chromosome 8 in two patients (cases 4 and 6) and a terminal deletion of chromosome 5 with breakpoint at band q31 in one patient (case 3). On the other hand, some chromosome aberrations, such as del(3)(q21) (patient 2, Fig. 2) and del(11)(q23) (patient 1, Fig. 3) presented a more selective distribution involving the megakaryocyte- and monocyte-enriched cell fractions, respectively.

DISCUSSION

Standard cytogenetic techniques do not permit recognition of the cytologic type of the dividing cells, thus causing a major problem in the analysis of myeloproliferative disorders where the involvement of a common progenitor stem cell gives rise to a multilinear hempoietic impairment. Recently, the introduction of a method for the simultaneous study of cell morphology and karyotype offered the chance to assign numerical chromosome aberrations to immunologically identified cell lines [12]. Alternatively, a chromosome analysis of enriched cellular fractions after separation on a density gradient might represent an effective contribution to a better

Table 3 Karyotypic patterns of enriched cell fractions in seven patients with CMMoL

| Patient	No. of cells	1070–1065 (6 hr)	No. of cells	1060–1050 (24–48 hr)	No. of cells	1050–1045 (24–72 hr)
1.	9	NN	6	NN	6	NN
	1	46,XX,del(5)(q31)	4	46,XX,del(11)(q23)	2	46,XX, − 10, + 21,del(5)(q31)
			2	46,XX, − 6, + 20	3	45,XX, − 16
2.	10	NN	9	NN	11	NN
			2	47,XY, + 21	4	46,XY,del(3)(q21)
					1	47,XY, + 21
3.	4	NN	8	NN	7	NN
	3	46,XY,del(5)(q31)	5	46,XY,del(5)(q31)	1	46,XY,del(5)(q31)
	2	47,XY, + 20,del(5)(q31)	1	46,XY, + 20, − 22,del(5)(q31)	2	44,XY, − 19, − 22,del(5)(q31)
	2	45,XY, − 15	2	46,XY,t(1;2)(q32;q36)	2	47,XY, + 14
4.	1	47,XY, + 8	2	47,XY, + 8	5	47,XY, + 8
	5	46,XY, + 8, − 22	5	45,X, − Y, + 8, − 22	2	50,XY, + 8, + 12, + 13, + 20
	2	46,X, − Y, + 8,del(20)(p12)	3	NN	3	NN
	3	45,XY, − 13				
5.	10	NN	8	NN	—	Inadequate sample
6.	4	46,XX, + 8, − 18	4	45,XX, + 8, − 18, − 20	2	47,XX, + 8
	2	47,XX, + 21	2	46,XX, + 8, − 18	1	NN
	3	NN	3	NN		
7.	9	NN	10	NN	7	NN

Abbreviation: NN, normal karyotype.
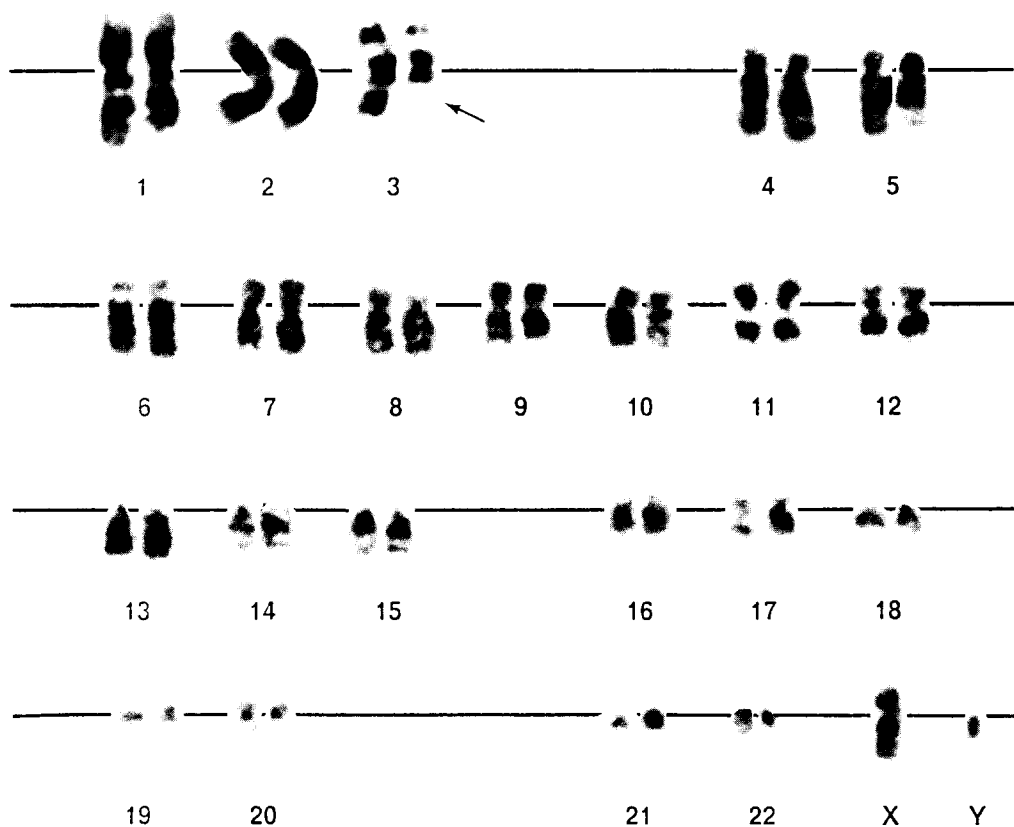
Figure 2   The karyotype obtained from 1050–1045 mg/ml megakaryocyte-enriched cell fraction (patient 2) is shown. The terminal deletion of chromosome 3 is arrowed.

definition of the cytogenetic aspects of the different hemopoietic lineages in this group of hematologic malignancies. As far as the cytogenetic results are concerned, our data parallel those given in previous reports [5, 13, 14]; nevertheless, some comments should be made regarding the methodologic approach. One patient (case 2) presented relevant dysmegakaryocytopoiesis with high platelet counts associated with a 3q21 aberration detected in metaphases preferentially arising in the 1050–1045 mg/ml fraction. Since normal karyotypes were exhibited by erythroid and immature myeloid cells, this abnormality seems to be confined to the megakaryocyte-enriched cell fraction. This clinical–cytogenetic association has been described in different myeloproliferative disorders [13, 15–18], suggesting the existence in this region of a regulatory locus for thrombopoiesis.

Abnormalities of chromosome 11 have been reported only occasionally in CMMoL and myelodysplasia [19, 20], whereas 11q23 rearrangements are considered typical of leukemic proliferations with monoblastic differentiation [21–23]. One patient (case 1) with hyperplastic features of the monocytic lineage and an increase of immature monocytes exhibited a clone carrying an 11q– abnormality among the metaphases arising in the monocytic cellular fraction. This cytogenetic finding is not surprising for a patient with a dysplastic monocytic lineage since its relationship with the differentiation of monocytes has already been pointed out [24, 25]. Interestingly, this abnormality was only present in the preparation harvested after 48 hours' incubation and was not detected after 72 hours' culture in the 1045–1050
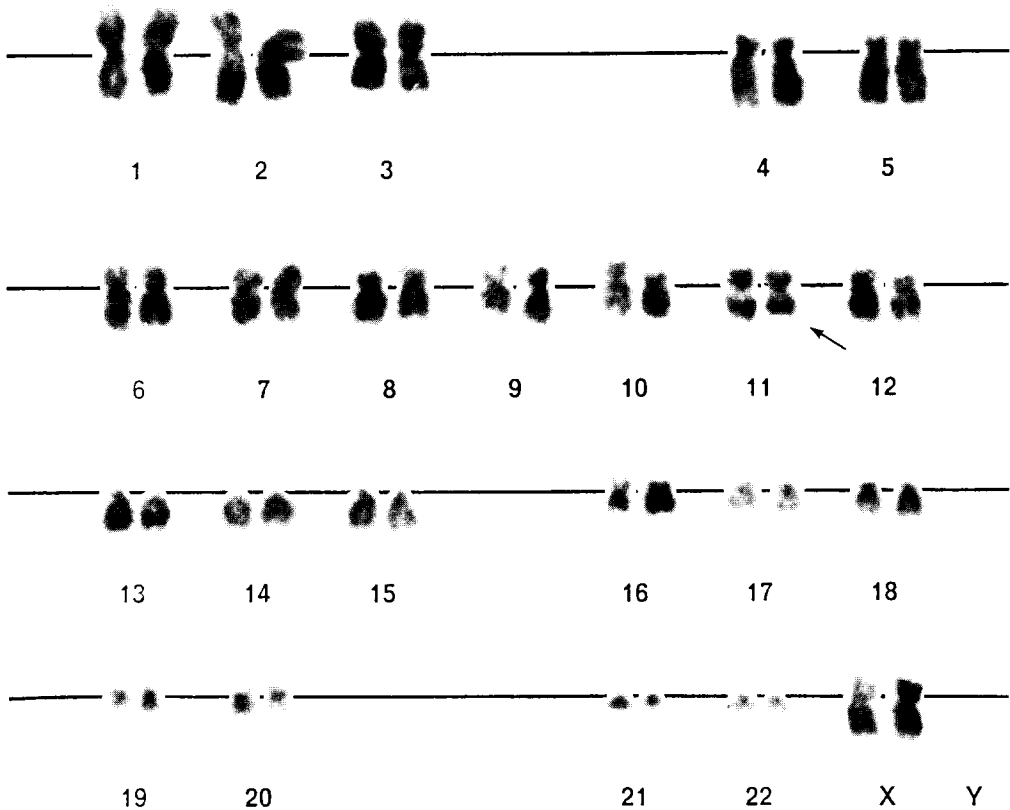
Figure 3 The karyotype obtained from 1060–1050 mg/ml monocyte-enriched cell fraction (patient 1) showing a terminal deletion of chromosome 11 is presented.

mg/ml fraction where 20% monocytic cells were present. This finding seems to emphasize the role played by culture time in cytogenetic analysis and gives evidence of the different mitotic activity of bone marrow cells during short-term culture [26]. It is thus likely that the kinetic features and low proliferative capacity that seem to characterize this clone [24] impede its detection in cytogenetic preparations obtained from whole bone marrow samples, possibly accounting for its absence in previous cytogenetic reports.

Some of the chromosome aberrations detected in our patients (+ 8,5q −) are typical of leukemic myeloid proliferations and have alrady been described in this myeloproliferative disorder. Their distribution appeared not to be correlated with the cytologic composition of the cellular fractions, thus providing cytogenetic evidence for the involvement of a common progenitor stem cell in this myelodysplastic syndrome [27]. It is conceivable that some steps in the progression of the disease might be represented by the occurrence, in either a multipotent or a committed hemopoietic progenitor, of genetic aberrations causing growth advantage or maturation impairment of single marrow cell lineages.

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