CONCISE REPORT

Growth and Cytogenetic Characteristics of Bone Marrow Colonies From Patients With 5q− Syndrome

By F. Carbonell, H. Heimpel, B. Kubanek, and T.M. Fliedner

Early erythroid precursor cells and myeloid progenitor cells (CFU-GM) from four patients with 5q− syndrome were cultured in order to study the in vitro growth patterns and to determine the clonal origin of this hematologic disorder. Cultures of CFU-GM exhibited normal colony growth, while erythroid progenitor cells demonstrated a marked decrease or absence of colony growth. Chromosomal studies indicate the 5q− chromosome is present in both hematopoietic progenitor cells, suggesting that the syndrome is an acquired clonal disease arising from a pluripotent hematopoietic stem cell. Follow-up cytogenetic studies reveal a decrease in the number of normal metaphases. This finding is consistent with reports that emphasize the slowly progressive nature of this malignant stem cell defect.

THE ASSOCIATION of an interstitial deletion of the long arm of chromosome 5 and refractory anemia, the 5q− syndrome, was described by van den Berghe et al[1] and Sokal et al[2] in 1974 as a further example of a close association between a chromosomal abnormality and a distinct clinical entity. It has been suggested that the chromosomal abnormality is acquired and probably originates from a pluripotent hematopoietic stem cell, but at present, only morphological arguments can be found in favor of this hypothesis.[1,2]

The present investigation was undertaken to characterize the in vitro growth patterns and to determine the clonal origin of the hematopoietic colonies in this hematologic disorder.

MATERIALS AND METHODS

Patients. The patients investigated between October 1977 and December 1983 at the University Hospital of Ulm were derived from a population of 47 cytogenetically examined cases, each with dysmyelopoietic syndrome. All patients signed a consent form allowing bone marrow biopsies to be performed.

Culture assays for hematopoietic colony-forming cells. Iscove's modified methylcellulose culture system was used as previously described.[3] Bone marrow was aspirated from the posterior iliac crest. Diluted EDTA–bone marrow cells were applied to a Percoll gradient (Pharmacia, Uppsala, Sweden) for cell separation and 10^5 bone marrow mononuclear cells were plated in 1 mL of Iscove's modified Dulbecco's medium (GIBCO, Grand Island, NY), containing 0.8% methylcellulose, 30% fetal calf serum, 1% deionized and delipidated bovine serum albumin, 360 μg human transferrin saturated with iron, and 10^{-4} mol/L alphathioglycerol. In addition, cultures for early erythroid precursor cells (BFU-E) were supplemented with 1 or 2 units of anemic sheep plasma erythropoietin Step III (Connaught Laboratories, Ontario, Canada) and stimulated with media conditioned by human peripheral blood leukocytes and/or irradiated blood mononuclear cells[3]; cultures for myeloid progenitor cells (CFU-GM) were stimulated with human placenta conditioned medium.[4] Duplicate cultures of CFU-GM and BFU-E were counted 8 days and 14 days, respectively, after incubation at 37 °C in saturated humidified air with 5% CO_2.

Cytogenetic studies. Bone marrow chromosomes were examined by the direct method. The cells were swelled with KCl 0.075 mol/L, fixed three times with methanol:acetic acid (3:1), spread on slides, and dried in air. To study the cytogenetic composition of hematopoietic progenitor cells, Colcemid (0.1 μg/mL) was added to each Petri dish and incubated for three hours. The colonies were collected and pooled in 5 mL of Hanks' balanced salt solution. Further processing followed standard techniques for chromosome preparations. Cytogenetic analyses of the phytohemagglutinin (PHA)-stimulated blood lymphocytes were done at day 3. Chromosome identification was obtained by the trypsin-Giemsa–banding technique.[5]

RESULTS

The clinical course and hematologic data of four patients at the time of cytogenetic investigation are presented in Table 1. All patients were characterized by macrocytic anemia and thrombocytosis. Granulocyte counts were borderline low or normal. The bone marrow samples (as estimated by bone marrow biopsy) were normocellular or moderately hypocellular. The most marked abnormality was the presence of hypolobulated megakaryocytes. They were smaller than normal megakaryocytes but not as small as the typical "micromegakaryocytes" of chronic myeloid leukemia. One patient (case No. 1) developed acute myeloid leukemia 93 months after the initial diagnosis of the dysmyelopoietic syndrome; at the end of the study period (March 31, 1984) the others had not.

The results from bone marrow chromosome studies and in vitro evaluation of growth patterns of bone marrow mononuclear cells are summarized in Table 2. The chromosomal abnormality in all four patients was interpreted as an interstitial deletion of the long arm of chromosome 5 [del(5)(q13;q33)]. Cases No. 1, 2, and 4 also had cells with normal karyotypes and cells with trisomy 8, but without the 5q− chromosome. Cytogenetic analyses of lymphocyte cultures showed normal karyotypes in all cases.

Cultures of the granulocytic–monocytic progenitor cells (CFU-GM) showed normal colony growth. The quantity and morphology of the colonies were similar to those obtained in a population of healthy bone marrow donors studied in our laboratory.[6] In contrast, the immature erythroid progenitor cells (BFU-E) were either absent or markedly decreased in

From the Departments of Clinical Physiology & Occupational Medicine, Internal Medicine III (Hematology-Oncology), and Transfusion Medicine, University of Ulm, FRG.

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Address reprint requests to Dr F. Carbonell, Department of Clinical Physiology and Occupational Medicine, University of Ulm, Oberer Eselsberg, D-7900 Ulm, FRG.

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Table 1. Clinical and Hematologic Data

Patient No.	Age/Sex	Blood				Bone Marrow				Survival (mo)	Evolution to Leukemia
		Hb (g/L)	MCV (fL)	Granulocytes ($\times 10^9$/L)	Platelets ($\times 10^9$/L)	Cellularity	E/M Ratio	Blast Cells (%)	Hypolobulated Megakaryocytes		
1	52/F	77	111	1.28	710	Normal	0.5/1	2	+	95	AML
2	40/F	82	104	1.50	414	Normal	0.8/1	7	+	>113	No
3	50/M	109	103	1.55	462	Normal	0.2/1	3	+	>51	No
4	73/M	99	119	5.77	858	Normal	0.3/1	8	+	>48	No

E/M, erythroid/myeloid; AML, acute myelogenous leukemia.

number. The cytogenetic studies performed on metaphases obtained from a pool of colonies indicated the presence of 5q− chromosomes in both types of hematopoietic progenitor cells.

In two of the cases, colonies with normal karyotypes could also be found. Due to the low number of colonies from which metaphases could be grown, it is not possible to exclude the additional presence of colonies derived from normal erythropoietic clones.

DISCUSSION

Most of the studies of hematopoietic progenitor cells in myelodysplastic syndromes have been reported on bone marrow CFU-GM. These studies have shown an abnormal growth pattern in a varied proportion of patients.[7-11] The data published about BFU-E or CFU-E also describe aberrant growth patterns with low or undetectable numbers of BFU-E.[9,12-15] However, normal to increased numbers of CFU-GM and CFU-E have also been found in these patients. The considerable overlap of cell culture data in these disorders could be explained by the biological heterogeneity of these diseases and by the methodological differences that are inherent in these nonstandardized culture systems.

Few data on the growth of hematopoietic precursors have been reported on the 5q− syndrome. Previous studies have shown a normal CFU-GM growth in 11 patients.[9,16,17] The only report on BFU-E of 5q− patients described a low colony-forming efficiency.[9] We were able to show that both granulopoietic and erythropoietic colonies can arise from the transformed clone. These data support the hypothesis that the 5q− syndrome is a clonal disease characterized by an abnormal cell line derived from a pluripotent hematopoietic stem cell. The results of cytogenetic follow-up studies suggest that the proportion of chromosomally normal metaphases decreases with the evolution of the disease. It appears that the 5q− syndrome is a slowly progressive malignant stem cell defect with a subsequent loss of normal stem cells.

Unfortunately, the number of cases and investigations in our study is not enough to draw a definitive conclusion, and more cases are needed to establish the clonal evolution of this syndrome. The clone with trisomy 8 was relatively stable during evolution of the disease, and we were not able to detect it in stem cell cultures. It may be speculated that clones with trisomy 8 were unable to differentiate to the stage recognized by line-specific colony formation.

With glucose-6-phosphate dehydrogenase isoenzyme as a marker, similar conclusions have been reported in patients with other myeloproliferative disorders, such as chronic myelocytic leukemia, polycythemia vera, essential thrombocythemia, and agnogenic myeloid metaplasia.[18,19] Although each of these diseases involves a multipotent hematopoietic stem cell, there are marked differences in clinical phenotype and pattern evolution. The mechanisms underlying these differences are unclear. The presence of specific chromosome aberrations in some of these diseases (such as Ph' chromosome-positive chronic myelocytic leukemia and the 5q− syndrome) and recent observations that some cellular oncogenes are localized near the breakpoints of affected chromosomes suggests an involvement of these genes in the generation of neoplasms.[20,21] Several observations support the existence of a functional relationship between growth factors and oncogene products.[22] The heterogeneity of the clinical and biological expression could be related to the different kinds of genetic alterations regulating the proliferation and differentiation of the hematopoietic stem cells.

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Table 2. Cytogenetic Data and Colony Growth Characteristics

Patient No.	Date	Bone Marrow Direct		Median No. of Colonies[a]		Colony Cytogenetic	
		No. of Metaphases	Karyotypes	CFU-GM	BFU-E	CFU-GM	BFU-E
1 A	4/81	6/44/10	N/5q−/+8	86	4.5	5q− (8)	5q− (2)
B	1/83	0/28/2	N/5q−/+8	76.5	3.6	5q− (17)	5q− (6)
2 A	5/81	24/16/0	N/5q−/+8	43	0	N(6)/5q− (5)	ND
B	5/82	3/30/0	N/5q−/+8	50	0	N(2)/5q− (7)	ND
3	8/81	0/32/0	5q−	21	2.2	5q− (11)	5q− (3)
4	3/82	4/30/1	N/5q−/+8	36	0	N(1)/5q− (6)	ND

(A) Colonies per 10^5 plated cells. Normal values for BFU-E and CFU-GM obtained in our laboratory with similar methods in a population of healthy bone marrow donors (10^5 nucleated bone marrow cells): CFU-GM, 32.5 ± 11.7; BFU-E, 43.2 ± 21.6. (B) The number in parentheses indicates the number of metaphases analyzed. N, normal karyotype. ND, not done.

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