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Phenotype of Early Erythroblastic Leukemias

By J.L. Villeval, P. Cramer, F. Lemoine, A. Henri, A. Bettaieb, F. Bernaudin, Y. Beuzard, R. Berger, G. Flandrin, J. Breton-Gorius, and W. Vainchenker

Nine cases of early erythroblastic leukemia, unidentified by usual criteria, have been diagnosed using a panel of antibodies. Three cases arose in patients with Down's syndrome, one in a patient with therapy-related leukemia, and four patients were in blast crisis of chronic myeloid leukemia; only one case arose de novo. Blast cells could be assigned to two main stages of erythroid differentiation: (1) presence of all erythroid-specific proteins in two patients, a phenotype corresponding to an immature erythroblast; (2) absence of the erythroid markers such as glycophorin A and spectrin in the presence of carbonic anhydrase isoenzyme I, ABH group antigens, and the antigen defined by FA6 152 monoclonal antibody in six patients, a phenotype related to a late erythroid progenitor (CFU-E). One patient had an intermediate phenotype. All patients except one demonstrated a megakaryocytic component. In three patients, chromosomal abnormalities were present, detected both in blasts and in erythroid colonies. In conclusion, these findings indicate that (a) most "cryptic erythroleukemias" are blocked at a "CFU-E–like" stage of differentiation, (b) it may be a frequent event in Down's syndrome and chronic myeloid leukemia, and (c) these erythroleukemias are phenotypically heterogenous.

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ON THE BASIS of conventional criteria, erythroleukemia represents an uncommon disease, ie, less than 5% of the leukemias. At least some of these leukemias may not be true erythroid neoplasms, since frequently the marrow becomes progressively invaded by myeloblasts.[1] In some cases of M_6 leukemia[2] the erythroblasts originate from residual normal marrow cells.[3] This low incidence of erythroleukemia in humans seems strange, since myeloproliferative diseases, especially chronic myeloid leukemia (CML), originate in a pluripotent stem cell.[4] The concept of "cryptic erythroleukemia" or of an "early" erythroblastic leukemia (leukemic cells blocked at an early stage of the erythroid differentiation and not morphologically identifiable by the usual criteria) has emerged. Most authors have used antibodies against glycophorin A (GPA) as a probe for the diagnosis of these "early" cases. Andersson et al[5,6] used a polyclonal antibody against GPA and suggested that "cryptic erythroleukemia" is frequent among M_1-acute myeloid leukemia (AML), relapse of acute lymphoblastic leukemia (ALL), and blast crisis of CML.[7] Other authors used several monoclonal antibodies (MoAbs) against GPA and concluded that erythroleukemia is uncommon.[8-11] This low incidence of erythroleukemia could be due to the fact that GPA detected by MoAbs was not a sufficiently early marker of erythroid differentiation. Indeed, GPA detected by these MoAbs is not expressed on the membrane of erythroid progenitors, ie, CFU-E and BFU-E.[12]

In the present study, we have investigated acute leukemia with a panel of MoAbs and polyclonal antibodies, including an anti-carbonic anhydrase I (CAI) and FA6-152 MoAb.[13] These two antibodies recognize antigens that are expressed before GPA during normal erythropoiesis.[13,14] Nine cases of erythroblastic leukemias could be diagnosed; the anti-GPA MoAbs only identified two of these cases.

MATERIALS AND METHODS

Leukemia samples. Over a period of 3 years, 310 cases of acute leukemia were investigated in our laboratory by immunological markers and by electron microscopy. Mononuclear cells were separated by Ficoll-metrizoate gradient density centrifugation (Lymphoprep, Nyegaard, Oslo, d:1077)[15] and subsequently investigated.

The nine cases presently reported were diagnosed among 120 cases of poorly differentiated acute leukemia. Patients 5 through 8 were identified as part of systematic investigations of the phenotype of CML blast crisis. The others (patients 1 through 4 and 9) were referred to our laboratory because they could not be classified according to the French-American-British criteria.[2] In two of these cases (patients 1 and 3), the erythroid origin of the blast cells was strongly suspected based on the morphological features, ie, the intensely basophilic cytoplasm.

Panel of antibodies and lectin. Details of antibodies used in this study are given in Table 1. During the first two years, the panel of MoAbs did not include early erythroid markers other than the LICR-LON-R10 MoAb[16]; During the last year, two new immunological markers that recognize early erythroid markers[13,14] were added to the previous panel. For blood group A patients, the A antigen was investigated using the Helix pomatia (HPA) lectin directly coupled to fluorescein (Industrie Biologique Française, Villeneuve la Garenne, France). Among 30 cases of non-erythroid leukemia, we have detected this antigen in only one case of megakaryoblastic leukemia.

The anti-CAI antibody and the FA6 152 MoAb were recently characterized using normal and leukemic cells.[13,14] Eighty representative cases of AML, ALL, or CML were screened with the anti-CAI antibody. CAI was not observed in non-erythroid hematopoietic cells with the exception of three cases in which this isoenzyme was detected in platelets. Further investigation of the occurrence of CAI in the megakaryocytic lineage has shown the absence of this enzyme in the platelets of 30 normal donors and 50 cases of myeloproliferative disorders. The FA6 antibody was used to screen more than 110 cases of AML, ALL, and blast crisis of CML. This antibody did not bind to ALL cells or M_1, M_2, and M_3 acute

From INSERM U.91, Hôpital Henri Mondor, Créteil, France; the Laboratoire de Cytogénétique, Institut de Recherches sur les Leucémies et Maladies du Sang, and Laboratoire Central d'Hématologie, Hôpital Saint-Louis, Paris; and Service de Pédiatrie Hôpital Intercommunal, Créteil, France.

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Address reprint requests to Dr William Vainchenker, INSERM U.91, Hôpital Henri Mondor, 94010 Creteil, France.

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Table 1. Antibodies Used

Designation	Monoclonal	Specificity	References
Granulomoncytic markers			
80 H 5	+	SSEA-1 antigen (Promyelocyte → polymorph) (Monoblast → promonocyte)	17
MO_2	+	Monocytes	18
Anti-My 7	+	Immature myeloid cells, monocytic series	19
Anti-My 9	+	Immature myeloid cells, monocytic series	20
Anti-myeloperoxidase	−	Myeloperoxidase (Myeloblast → polymorph) (Monoblast → monocyte)	21
Platelet markers			
AN 51	+	Platelet glycoprotein Ib (Promegakaryoblast → platelet)	22
J 15	+	Glycoprotein IIb-IIIa? (Promegakaryoblast → platelet)	23
5 G 11	+	Thrombospondin (Promegakaryoblast → platelet)	24
Anti vWF	−	vWF (Promegakaryoblast → platelet)	Dako (Copenhagen) 25
B Cell markers			
B 1	+	Pre B → lymphocyte	Coulter clone (Hialeah, Fla) 26
J 5	+	Common ALL antigen	Ortho (Westwood, Mass) 27
T cell marker			
T 11	+	E rosette receptor	Ortho (Westwood, Mass) 28
Progenitor markers			
20-6	+	HLA-DR	29
Anti-My 10	+	All progenitors except CFU-E Immature myeloid cells	30
Erythroid markers			
LICR-LON-R10 and R 18	+	Glycophorin A (Proerythroblast → erythrocyte)	16
Anti-glycophorin A	−	Glycophorin A	5
LICR-LON-R6A	+	Band 3 ? (Erythroblast → erythrocyte)	16
	−	Band 3	
CA 5	+	β Chain of spectrin (Proerythroblast → erythrocyte)	
	−	α and β Chain of spectrin	
FA6 152	+	A part of BFU-E, CFU-E → erythroblast Megakaryocyte → platelet, monocyte	13
Anti-Gerbich	+	(CFU-E → erythrocyte), glycophorin C	Centre National de Transfusion Sanguine (Paris) 31
Anti-globin chain	−	γ-Globin chain	32
Anti-hemoglobin	−	Hemoglobin	Cappel (Cochranville, Pa)
Anti-CAI	−	CAI (CFU-E → erythrocyte)	14

leukemia cells. Positive cells were identified in leukemias with a monocytic component such as some M_5 and M_4 and part of the megakaryocytic leukemias. In all these cases, the monocytic markers recognized by 80H5,[17] My7,[19] My9,[20] anti-HLA-DR[29] MoAbs, or the megakaryocytic markers identified by AN51[22] or J15[23] MoAbs were detected on these non-erythroid FA6-positive cells.

Indirect immunofluorescence. Leukemic cells to be characterized were used fresh or thawed when further investigation was needed. An indirect immunofluorescence technique, including double-staining experiments, was used on fixed or unfixed cells.[14] For routine immunodiagnostic procedure, the panel of surface markers listed in Table 2 was used as well as three antibodies recognizing cytoplasmic proteins, ie CAI, myeloperoxidase (MPO), and von Willebrand factor (vWF). When the phenotype was compatible with that of an erythroid precursor (positive cells with FA6 MoAb, the anti-CAI antibody, and in some with anti-GPA MoAbs), the other erythroid markers listed in Table 3 were also evaluated.

Acetylcholinesterase. Acetylcholinesterases (AChE) were cytochemically revealed by the technique of Jackson.[33]

Clonogenic assays. Clonogenic assays for the different types of hematopoietic progenitors were performed by the methylcellulose techniques.[34] The stimulating factors were 1 IU/mL porcine Epo (26

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Table 2. Cell Marker Results

Patient No.	Primary Syndrome	Age (yr)	Sex	Leukocytes (/μL)	Blasts	Cells Staining (%) 20-6	My 10	My 9	My 7	80 H 5	MO_2	B1	J5	T11	AN 51	J15	FA6 152	R 10 R 18
1	Down's syndrome	3	M	15,000	65	1	<1	<1	<1	<1	<1	<1	0	11	3	7	70	58
2	Down's syndrome	2	M	12,000	60	15	0	ND	0	1	1	0	0	23	3	3	66	1
3	Down's syndrome	1.5	M	10,000	50	3	0	<1	<1	<1	3	8	0	14	0	0	70	0
4	Therapy-related leukemia (Hodgkin's disease)	49	M	6,800	50	ND	ND	ND	ND	8.5	ND	ND	0	0	19	22	ND	26
5	CML	34	M	*	63	2	4	ND	<1	<1	<1	0	0	2	3	3	70	6
6	CML	58	F	103,000	19	0	0	0	0	54	0	0	0	0	2.5	3	46	4
7	CML	17	M	82,000	23	5	5	4	5	42	0	0	0	<1	16	17	52	<1
8	CML	50	F	71,000	56	0	0	0	0	0	0	<1	0	2	1	13	78	3
9	—	58	M	52,000	47	2	2	2	2	7	2	1		2	1	1	70	<1

The results are expressed in percentage of fluorescent-labeled cells isolated by Ficoll-metrizoate density centrifugation. The percentage of blasts was determined on the May-Grünwald-Giemsa on standard smears. In patients 6 and 7, the Ficoll-metrizoate density centrifugation has led to a twofold enrichment in blast cells as a consequence of the presence of numerous mature myeloid cells. ND, not determined; *, marrow specimen.

IU/mg of protein, Centre National de Transfusion Sanguine, Paris) and phytohemagglutinin-leukocyte–conditioned medium (PHA-LCM).[35]

Cytogenetics. Chromosome analysis was carried out on unstimulated blood cells and bone marrow cells after short-term culture (24 to 48 hours) in all cases and on colonies in three cases (5, 6, and 9). In addition, chromosome analysis was carried out after 2 months in liquid culture in patient 6. R bands using Giemsa after heating (RHG) and G bands using Wright staining were performed, and the international nomenclature[36] was used for the classification of the chromosomes. Metaphases from individual colonies were analyzed for three patients using a slight modification of the technique of Dubé et al.[37]

RESULTS

Immunofluorescence assays. Among 120 cases of leukemias, nine cases had a major erythroid component. In two of these cases (patients 1 and 4), the diagnosis could be made with anti-GPA MoAbs. In the seven others, the diagnosis depended on demonstration of cells that bound the FA6 MoAb and the anti-CAI antibody. Only 0% to 6% of cells from these patients were labeled by the anti-GPA MoAbs (Table 2).

In order to accurately determine the phenotype of the blasts, the nine leukemic samples were further investigated with the other erythroid markers (Table 3). Patient 4 exhibited two populations of blast cells distinguished by their size. The very large GPA- and CAI-positive blasts also contained HbF, whereas the small blast cells expressed platelet glycoproteins and vWF, suggesting an origin from the megakaryocytic lineage. In patient 1, the blast cells exhibited nearly all erythroid markers.

Among the seven other patients (patients 2, 3, and 5 through 9), one (patient 2) exhibited a peculiar phenotype. AChE, HbF, and glycoprotein band 3 were detected in the same cell population identified by the GPA MoAbs. In contrast, the anti-GPA polyclonal antibody bound to 40% of the blast cells. In addition, spectrin and Gerbich and A antigens were detected in the majority of these blasts. The other six patients (3 and 5 through 9) had a somewhat heterogenous phenotype. Only Gerbich or A antigen was clearly expressed in four cases. In the three blood group A patients (patients 3, 7, and 9), HPA lectin bound to about half of the leukemic cells, and in patient 8, Gerbich antigen was expressed in the same proportion of cells.

Double-labeling experiments were performed in four patients with CML blast crisis. In patients 6 and 7, a marked myeloid component was present but only included mature myeloid cells easily identifiable by light microscopy. In all cases, the myeloid and megakaryocytic cells, respectively labeled by 80H5, My9, 5G11, or J15 MoAbs, did not express

Table 3. Erythroid Cell Marker Results

Patient No.	FA6 152 (%)	GPA MoAb (%)	GPA Poly Ab (%)	Spectrin MoAb (%)	Spectrin Poly Ab (%)	R6A	BAND 3 Poly Ab (%)	CA I (%)	Hb F (%)	AChE (%)	Gerbich Antigen (%)	A Antigen
1	70	58	82	83	83	5	45	90	3	5	60	80
2	66	1*	35*	70*	75*	0*	1*	87*	0	0	60	90*
3	70	0	4	1	4	0	1	73	1	1	0	30
4	ND	26	ND	ND	ND	ND	ND	26	23	ND	ND	†
5	70	6	8	2	8	3	3	91	<1	5	4	†
6	46	4	4*	1*	4*	ND*	4*	62*	5*	4*	ND	†
7	52	<1	<1	ND	5	ND	1	25	1	1	<1	35
8	78	3	4	ND	5	0	2	62	<1	<1	30	†
9	70	1	4	<1	11	<1	ND	80	<1	<1	ND	34

The results were determined after fluorescent labeling except for the investigation of acetylcholinesterase (AChE), which was revealed by a histochemical technique. All samples were blood cells with the exception of patient 5, from whom marrow cells were obtained.

*These results have been determined from a second sample, obtained a few days later; for patient 6, in this second sample, 23% of the cells expressed MPO and 7%, vWF.

†These patients did not belong to blood group A.

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CAI. All 80H5-positive cells expressed MPO. Labeling with the antithrombospondin (5G11) and the anti-vWF was only observed in cells stained by J15. This labeling resulted in a granular appearance, suggesting that most of these cells were hypoploid mature megakaryocytes (micromegakaryocytes).[25] In addition, platelets from patients (3, 6, 7, and 9) were examined for their content of CAI; this isoenzyme could not be detected.

Finally, it is noteworthy that in all cases presently described, a constant lack of labeling was observed with the My9, My10, and the anti-HLA-DR MoAbs. In patient 2, the anti-HLA-DR MoAb stained 15% of the cells, but binding did not correspond to erythroid cells because CAI-positive cells were not stained by the anti-HLA-DR antibody in double-labeling experiments.

Morphological characterization. The morphology of these blasts was reexamined after diagnosis with the immunological markers. In all patients, erythroid blast cells had a peculiar and identical morphology (Fig 1B). They were characterized by a medium to large size (15 μ), an extremely basophilic cytoplasm with the exception of a zone localized near the nucleus, and the presence of several peroxidase-negative azurophil granules or small vacuoles in the Golgi zone. The nucleus was large with one or two nucleoli.

Karyotypic analysis in short-term cultured cells and erythroid colonies. Using standard short-term cultures, clonal acquired chromosomal abnormalities were observed in all patients except patient 3, in whom only a constitutional trisomy 21 was found. Karyotypic studies of colonies derived from clonogenic assays were obtained in patients 5, 6, and 9. In patient 5, erythroid colonies were formed. All mitoses of leukemic cells were hyperdiploid (56 to 64 chromosomes) in 24-hour blood cell culture. All had a Philadelphia (Ph^1) chromosome and extra chromosomes with some cell-to-cell variation. A similar pattern of chromosomal changes was found in erythroid colonies from bone marrow, but only diploid mitoses with Ph^1 chromosome were found in other colonies, except one cell in a blood granulomonocytic colony (Table 4). In patient 9, no granulomonocytic colonies could be grown. Erythroid colonies comprising up to 400 cells were obtained in the presence or absence of added Epo. The cells composing these colonies expressed all the specific erythroid proteins, including hemoglobin. Multiple chromosomal abnormalities were found in unstimulated 48- and 78-hour blood cell cultures and in erythroid colonies except in four mitoses without markers (Table 4). A marker ? t (1;15) (p.21; q36) was present in all abnormal mitoses either from short-term blood cultures or from erythroid colonies. Other abnormalities, such as monosomies 17 and 10, were also common but not always observed, with some variation from one cell type to another. The majority of the cells were hypodiploid.

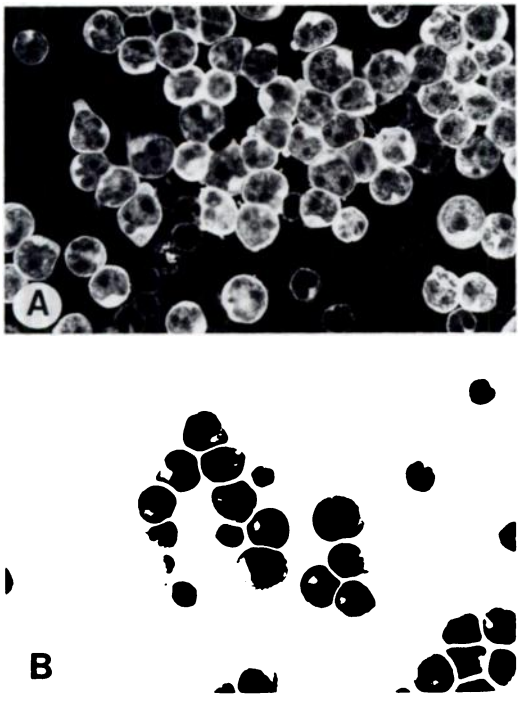

Fig 1. Blast cells from patient 2. (A) Labeling with the anti-CAI antibody. A marked staining is observed in all blast cells. (B) Morphology of the blast cells after May-Grünwald-Giemsa staining. The blasts are characterized by a medium size, an extremely basophilic cytoplasm, and an often irregular nucleus with one or two large nucleoli.

In patient 6, two types of erythroid colonies were obtained in the presence of Epo, either "morphologically normal" erythroid colonies similar to BFU-E colonies or atypical colonies comprising dispersed cells. The erythroid nature of these colonies could be demonstrated by the presence of CAI, HbF, and GPA in the cells by immunofluorescence labeling. In the absence of added Epo, only atypical erythroid colonies were obtained. Karyotypic analysis revealed two types of mitosis in unstimulated 24-hour blood cell cultures as well as in erythroid colonies (Table 4). Some had the Ph^1 chromosome as the only abnormality; the others had 48 to 64 chromosomes, including the Ph^1 chromosome; the representative karyotype of these cells was 53XX, +1, +3, +6, +10, +21, 22q−, t(9;22), 17p+.

The distinction between the morphologically abnormal erythroid colonies and the granulomonocytic colonies was difficult. In the first set of experiments, nearly all the morphologically presumed granulocytic colonies were in fact erythroid when studied with markers of differentiation. In a second set of experiments, we excluded the more dispersed colonies from plucking. Studies with MoAbs showed that 75% of the harvested colonies were indeed granulomonocytic, but some were erythroid; this could explain the finding of six hyperdiploid mitoses in granulomonocytic colonies. In the presence of Epo, some "morphologically normal" erythroid colonies of patient Val only had the Ph^1 chromosome. Finally, blood cells from patient 6 were grown in suspension for 2 months in the presence of a supernatant of the Mo-cell line.[39] More than 99% of the cells were granulocytic precursors at this time. Karyotypic analysis of these cells showed only the presence of a Ph^1 chromosome.

DISCUSSION

The nine cases presently described were found in a sample of 120 leukemic samples that could not be classified by

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Table 4. Cytogenetics
Number of Metaphases Studied

Material*	Number of Chromosomes																						
	≤43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Case 6‡																								
B 24 h			7	2		2				4	6	3	5	3	1									
G M colonies (B)	2	5	4	5			1			1		1		1								1		
E colonies (B)		1	2	1					1	3	3	1	2	2	1									
2 month liquid culture (B)	2	1	1	2																				
Case 5‡																								
B 24 h														1	1	1	2	1	1	2	1	1		
E colonies (M)															1	1	3			3	5	3	1	1
E colonies (B)			1	3																				
G M colonies (M)			1	6																				
G M colonies (B)			3	9										1										
Case 9§																								
B 48 h	9	5	2°	2°																				
B 72 h	8	5	1°	5	2																			
E colonies	4	5°	9	1																				

Erythroid colonies were collected in cultures with Epo, GM colonies were grown with Mo-medium, PHA-LCM, or Mo-medium + Epo. °, 1 mitosis without marker; G M colonies, granulomonocytic colonies; E, erythroid; B, blood; M, bone marrow.

*M 48 h, B 24 h, B 48 h, B 72 h cultures of bone marrow for 48 hours, of blood for 24 hours, 48 hours, and 72 hours, respectively, without mitogens.

‡All metaphases with Ph^1 chromosome.

§All metaphases abnormal but not all identical.

morphological and cytochemical criteria. Their phenotype differs from all other cases (see Table 5)[40] by the constant expression of both CAI and the antigen identified by the FA6 MoAb. In addition, the morphology of the blast cells from these cases was similar in many ways and was characterized by extremely basophilic cytoplasm and a large nucleus with one or two large nucleoli. Ultrastructural investigation of these nine cases (to be reported) has shown ferritin molecules located in peculiar cytoplasmic granules in the absence of rhopheocytosis vesicles. We have recently detected cells with the same morphology and the same ferritin-containing granules in a highly enriched CFU-E fraction obtained from normal marrows.

Investigation with other erythroid markers listed in Table 3 led us to distinguish two main phenotypes that correspond to discrete stages of the normal erythroid differentiation (Fig 2):

(*a*) In patients 1 and 4, the phenotype is identical to that of an immature erythroblast. The main erythroid proteins are detected, including GPA identified by MoAbs.

(*b*) In six patients (patients 3 and 5 through 9), the phenotype is related to that of a late erythroid progenitor. Leukemic cells are identified only by the early markers (CAI, FA6, Gerbich and A antigens), which are expressed by the normal CFU-E.[13,14,31,41] HLA-DR antigen is not detected on the surface of these leukemic cells and has been demonstrated to be absent or expressed at low level by normal CFU-E.[41-44]

Leukemic cells from patient 2 have an intermediate phenotype between these two groups because in addition to the early markers, spectrin and GPA (detected by a polyclonal antibody) were present. This last result suggests that GPA is synthesized in this patient in an incompletely-*O*-glycosylated form.[45]

The erythroid origin of these leukemic cells was further sustained by the presence of the same karyotypic abnormalities in the blast cells and in the erythroid colonies obtained in three of these patients. It is noteworthy that in these three cases (patients 5, 6, and 9), cells composing the erythroid colonies have acquired the main erythroid proteins, such as Hb, whereas the blast cells were devoid of them. However, these colonies obtained with an unusually high plating efficiency were abnormal by their poor hemoglobinization and by their growth in the absence of added Epo. In patient 5, the karyotypic abnormalities added to the Ph^1 chromosome were present in the erythroid colonies but absent from the granulo-

Table 5. Correlation Between Immunological Phenotype and FAB Classification for Leukemia

Leukemic Subtype According to FAB Classification		Reactivity With Panel of Antibodies																
		CAI	MPO	vWF	20-6	My 10	My 9	My 7§	80 H 5	MO_2	B1	J5	B4	T11	AN 51	J15	FA6	R10 R18
Myeloblastic	MO*, M1	0	+	0	+	+	+	+/−	+/−	0	0	+/−	0	+/−	0	0	0	0
	M2, M3	0	+	0	0	0	+	+/−	+	0	0	0	0	+/−	0	0	0	0
Monocytic	M4	0	+	0	+	0	+	+/−	+	+	0	0	0	0	0	0	+	0
	M5	0	+	0	+	+/−	+/−	+/−	+	0	0	0	0	0	0	0	0	0
Megakaryoblastic	M7⁺ mat[38]	0	0	+/−	+/−	0	0	0	0	0	0	0	0	+/−	+	+	+/−	0
	M7⁺ immat[38]	0	0	0	+	+	+	?	+/−	0	0	0	0	0	0	+/−	0	0
Erythroblastic	M6 (eryth. component)	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+

Only the major cellular component is represented in this table. In some leukemic subtypes, other minor cellular components are frequently observed as megakaryoblasts in the M_0 and M_1 subtypes, myeloblasts in the M_7 and M_6 subtypes, or erythroblasts in the M_7 subtype. The lymphoid lineage-associated antigens identified by the J5 and T11 MoAb are expressed in some AML. This table is based on immunodiagnostic investigation of 310 cases of acute leukemia and blast crisis of CML.

*Cytochemistry for myeloperoxidase was negative by light microscopy; however, these cases exhibited rare small granules containing myeloperoxidase at the ultrastructural level and have been called MO. This leukemic subtype is often labeled by the 80H5 MoAb in contrast to the M_1 subtype.

†Revelation of platelet peroxidase was performed in these cases. The anti-vWF and the FA6-152 MoAb are less sensitive than the J15 and AN51 MoAbs to detect M7 mature leukemia.

§The antigen recognized by the My 7 MoAb is related to the cellular proliferation.

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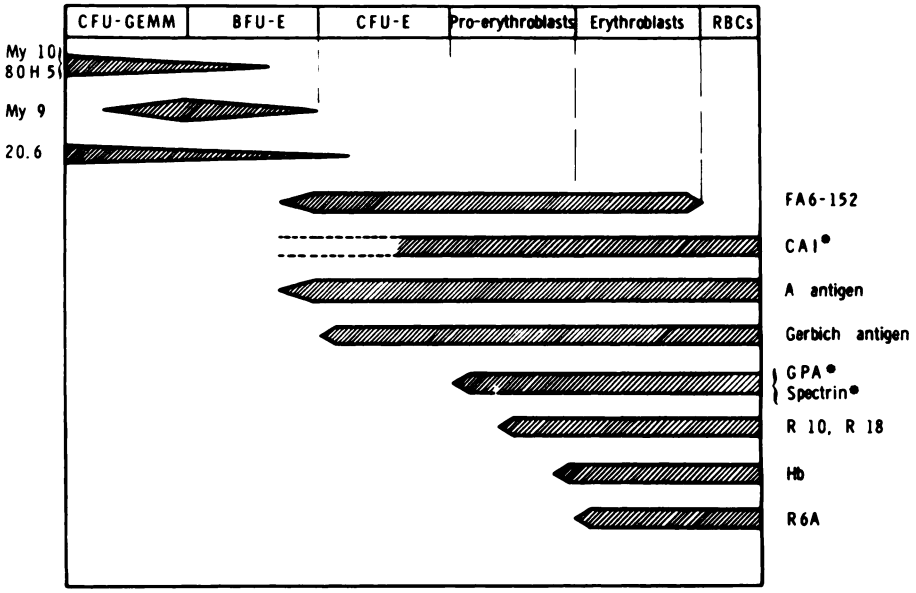

Fig 2. Pattern of the antigenic distribution-defined erythroid cell phenotypes during adult differentiation. The My7, Mo_2, B_1, J5, T_{11}, AN51, J15, and B_4 MoAbs–related antigens have not yet been detected during erythroid differentiation. •, antigens detected by polyclonal antibodies.

monocytic colonies with the exception of one ambiguous colony. The same result was obtained in patient 9, but we could not definitely separate the granulomonocytic from the erythroid colonies. However, in long-term suspension culture, the granulocytic lineage could be grown in the absence of erythroid precursors. In this case, the cells exhibited only the Ph^1 chromosome.

The phenotypes of these cases of erythroleukemia differ from those of the human erythroleukemic cell line K562, which expresses GPA and Hb[46-48]; however, K562 cells are devoid of CAI,[14] ABH antigens, and the antigen identified by FA6.[13] These nine cases are also apparently different from previously described cases of AML or ALL, in which some cells were doubly labeled by a polyclonal antibody against spectrin and the OKM_1 MoAb. From this result, it was suggested that leukemic myeloblasts may abnormally express erythroid-specific genes.[49] However, neither of these markers is truly lineage specific. Spectrin and spectrin-related proteins have been detected in non-erythroid cells,[50] including the lymphoid lineage.[51] The OKM_1 MoAb that recognizes the C_3b_i receptor[52] binds to cells of the granulomonocytic lineage and to natural killer and some lymphoid cells.[53] In addition, OKM_1 MoAb has recently been shown to bind to normal CFU-E.[54]

Erythroleukemia appears to be uncommon among de novo leukemias (about 1% to 2% in this study), whereas it may be much more common in blast crisis of CML and in Down's syndrome.[7-11,55] Among the 120 studied patients, erythroleukemia represented four of 30 cases of CML blast crisis and three of ten cases of Down's syndrome leukemias.

In conclusion, erythroleukemia and erythroleukemic blast crisis of CML appear to be heterogenous. Most correspond to the CFU-E–like stage of the normal erythropoiesis. They may be an unique source of material with which to investigate the molecular mechanisms regulating human erythropoiesis.

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