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Morphologic, immunologic and cytogenetic studies in erythroleukaemia: evidence for multilineage involvement and identification of two distinct cytogenetic-clinicopathological types

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Summary. Clinical features, as well as morphology, immunophenotype and cytogenetics were retrospectively studied in 20 patients with an original diagnosis of erythroleukaemia (EL) reclassified according to the FAB criteria. Fifteen patients had *de novo* EL, five patients had therapy-related EL. Myelodysplasia preceded the onset of EL in eight cases and myelodysplastic features involving multiple haemopoietic lineages were observed at leukaemia presentation in all cases. Immunologic findings confirmed multilineage involvement, showing sub-populations of cells expressing platelet-associated markers in more than 50% of cases tested and the presence of a myelomonocytic component, besides glycophorin A-positive cells. Cytogenetically, major karyotype aberrations (MAKA), defined by the presence of three or more aberrant events in the same clone, were observed in 14 cases, minor karyotype aberrations (MIKA) were observed in four cases and normal karyotype in two cases. No differences in the cytological-cytogenetic picture of our patients with *de novo* EL and with therapy-related EL were found suggesting that aetiological factors and/or pathogenetic mechanisms common to EL and secondary leukaemia may exist. All patients with MAKA had leftward shift of erythropoiesis with proerythroblasts and basophilic erythroblasts usually representing more than 50% of all erythroid cells. In patients with MIKA or normal karyotype, maturation of erythroid cells, though morphologically abnormal, was quantitatively preserved and early erythroblasts never exceeded 25% of erythroid cells. Clinically, the haemoglobin level at presentation, as well as in the proportion of patients achieving complete remission after chemotherapy, appeared to be lower in the maturation arrest-MAKA group as compared to the preserved maturation-MIKA/normal karyotype group. Median survival was shorter in the former group (3·5 months) than in the latter (median 13 months). Morphologic–immunologic–cytogenetic studies thus allow for the identification of two distinct cytogenetic-clinicopathological types of EL.

Leukaemias of erythroid lineage represent infrequent haematologic disorders (Roggli & Saleen, 1982) presenting heterogeneous cytological features. Classically, two pictures were distinguished, namely 'pure erythraemic myelosis' in which abnormal proliferation appeared to be mostly confined to the erythroid series, and 'erythroleukaemia' where myelomonocytic blast cells were also present (Dameshek, 1969). Transformation of the former clinical picture into the latter, as well as evolution into acute myeloid leukaemia (AML) were also described.

Variable biological features of erythroleukaemia have been reported in previous papers. Cytogenetic studies disclosed different karyotypic patterns, ranging from normal diploidy to major karyotype aberrations (MAKA) (Sandberg, 1980; Stamberg, 1987; Nakamura, 1989) and several reports, while consistently showing myelomonocytic involvement, have yielded conflicting results as to whether erythroblastic proliferation in erythroleukaemia should be considered malignant or not (Inoue *et al*, 1975; Bernheim *et al*, 1983; Suda *et al*, 1986). Thus it has been suggested that erythroleukaemia may represent, in some cases, AML with reactive

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erythroid hyperplasia (Freedman *et al*, 1986).

Heterogeneity also on clinical grounds stands out from recent literature (Hetzel & Gee, 1978; Griesser & Horney, 1987), where the lack of uniform diagnostic criteria hampered comparison among patients of different series, resulting in an inability to sharply define disease entities in this variegated neoplastic disorder.

Hence two issues of primary importance in our understanding of erythroleukaemia appear to be still controversial: (a) the presence of multilineage involvement, and (b) the definition of disease subsets in the spectrum of clinical manifestation of this myeloid neoplasia.

The introduction of the FAB proposals for the identification of erythroleukaemia (Bennett *et al*, 1985) has overcome the problem of lack of uniformity in diagnosis and, indeed, the value of the FAB classification of AML has been acknowledged, these proposals having provided simple and highly reproducible diagnostic criteria. Furthermore, this cytologic classification can be integrated with immunologic and cytogenetic findings (Second MIC, 1988) which represent important tools for a better definition of biological characteristics and prognosis of AML (Vaughan *et al*, 1988; Castoldi *et al*, 1989; Griffin *et al*, 1986; Keating *et al*, 1987).

For these reasons we undertook a retrospective study of patients with erythroleukaemia as defined by the FAB group, in which immunologic and cytogenetic data were available. Cytological features, as well as results of chromosome analysis and immunophenotyping are described and commented on in this report insofar as they relate to the issue of multilineage involvement in this haemopoietic neoplasia. The contribution of combined morphologic–immunologic–cytogenetic studies to the nosology of erythroleukaemia is also discussed.

PATIENTS AND METHODS

Twenty patients with a diagnosis of erythroleukaemia, referred to the Genetic Centre, Leuven, and the Department of Haematology, Ferrara (nos. 7 and 13), are included in the present report. Immunologic and cytogenetic data were available in all cases. The patients were retrospectively classified according to the FAB criteria (Bennett *et al*, 1985). All patients had more than 50% of erythroblasts in the bone marrow (BM). In 18 cases more than 30% of the remaining non-erythroid cells were blasts, thus fulfilling the criteria for the diagnosis of AML-M6. Two patients (nos. 11 and 18) showed respectively 21% and 27% blasts among non-erythroid cells and were classified as myelodysplastic syndrome (MDS) with major erythroid component. The whole group of patients will here be referred to as erythroleukaemia (EL).

(a) *Cytological studies.* Besides recommended procedures for the diagnosis of AML-M6, differential counts were made on each patient to assess the degree of leftward shift of erythropoiesis which was arbitrarily defined by the presence of more than 50% proerythroblast and basophilic erythroblast. Cytochemical stains were also reviewed to characterize the blast cell population. The presence of associated myelodysplastic features was evaluated in each patient. According to previously proposed criteria (Brito-Babapulle *et al*, 1987), trilineage myelodysplasia (TMDS) was defined by the presence of more than 25% dysplastic erythroblasts and more than 50% abnormal granulocytes and megakaryocytes.

(b) *Immunophenotyping.* Immunologic studies were routinely performed on BM mononuclear cells by indirect immunofluorescence technique. AB serum at a dilution of 2% was used to minimize non-specific binding to Fc receptors. Immunofluorescence staining was evaluated on at least 200 cells under a fluorescence microscope. Commercially available monoclonal antibodies detecting the following surface markers were used: myeloid associated antigens: CD34, CD33, CD13, CD14, CD15, CD41; lymphoid associated antigens: CD10, CD19, CD2, CD7. Anti Glycophorin A (GPA) monoclonal antibody was used in 12 patients. In four patients only a minority of markers had been tested. Terminal deoxynucleotidyl transferase (Tdt) activity was assayed in five patients by an indirect immunofluorescence technique using rabbit anti-calf Tdt serum (Bethesda Research Laboratories, Md.).

(c) *Cytogenetic studies.* Chromosome analysis was performed at leukaemia presentation in all patients on BM cells. Synchronization with methotrexate and bromodeoxyuridine was carried out. Metaphases were R-banded with acridine orange. At least 10 karyotypes were studied in each patient. Karyotypes of the different patients were grouped into normal, minor karyotype aberration (MIKA) and MAKA. MIKA was defined by one or two events of non-disjunction or translocation. MAKA was the result of three or more such events.

RESULTS

(a) *Clinical findings*

Histories of our patients were negative both for hereditary haematologic diseases and for acquired disorders inducing secondary dyserythropoiesis. Normal dietary habits were recorded in all cases. Fourteen patients (nos. 1–10, 12–15) presented with *de novo* AML-M6, whereas in four patients (nos. 16, 17, 19, 20) leukaemia was preceded by cytotoxic treatment of Hodgkin's disease (nos. 17, 19), of multiple myeloma (no. 16) and of polycythaemia vera (no. 20). Two patients (nos. 11, 18), one of which had multiple sclerosis treated with cyclophosphamide for 6 years, had MDS with a major erythroid component according to the FAB classification. However, left-shifted erythropoiesis with normoblasts and blast cells in the peripheral blood, and clinical features including hepatosplenomegaly, suggested the diagnosis of erythroleukaemia was more appropriate in these patients.

A myelodysplastic phase preceding the onset of EL was detected in eight patients, of whom three had therapy-related leukaemia. Duration of myelodysplasia ranged between 4 and 24 months (median 7 months).

Clinical features at leukaemia presentation in this series parallel previously reported findings. Patients' ages ranged between31 and 81 years, median 60 years. Anaemia related symptoms were recorded in all cases, fever in eight cases, petechiae, ecchymoses or bleeding in five cases. Physical examination revealed hepatosplenomegaly and lymph node enlargement in 12 and two cases, respectively. Chemo-

Table I. Clinical and haematologic characteristics in 20 patients with EL

Patient sex/age	Diagnosis	Toxic exposure	Hb (g/dl)	WBC ($\times 10^9$/l)	% bls (PB)	E bls (PB) ($\times 10^9$/l)	Platelets (10^9/l)	Treatment/ outcome	Survival* (months)
(1) F/60	M6	NE	5·4	15·8	9	4·9	54	HD-Ara-C/NR	7
(2) M/62	M6	NE	7·7	3·3	5	0·7	83	DNR+Ara-C/NR	4
(3) M/32	M6	Solvents	4·8	1·6	2	NA	163	DNR+Ara-C/CR	7
(4) M/72	M6	NE	6·7	9·5	68	4·6	39	DNR+Ara-C/NR	1
(5) M/51	M6	Solvents	8	2·8	1	<0·1	92	DNR+Ara-C/CR	10+
(6) M/60	M6	Solvents	7·1	1·6	21	0·4	47	DNR+Ara-C/NR	2
(7) M/56	M6	Pesticides	7·1	1·7	18	0·8	28	DNR+Ara-C/NR	2
(8) F/81	MDS→M6	Unknown	10·4	5·2	4	0·9	63	No therapy	4
(9) M/67	MDS→M6	Thorotrast†	9·9	4·4	18	0·25	36	HU/NR	4
(10) F/80	MDS→M6	Unknown	6·1	4·1	1	0·8	42	No therapy	4
(11) M/53	M6‡	Solvents	10·8	3·4	3	11	140	DNR+Ara-C/CR	10
(12) M/43	M6	NE	10·3	10·2	3	2·3	96	DNR+Ara-C,+ VP16+m-Amsa/CR	17+
(13) F/72	M6	Pesticides	8·1	11	45	0·3	35	No therapy	1
(14) M/31	MDS→M6	NE	8·5	4·8	10	0·5	54	VCR, DNR, Ara-C BMT/CR	12
(15) M/73	MDS→M6	NE	10·3	2·4	2	<0·1	64	No therapy	13
(16) M/50	t-M6	MPH	8·4	6·1	4	0·3	76	No therapy	2
(17) M/55	t-MDS→M6	PCZ	5·4	1·8	<1	<0·1	72	6-MP, HU/NR	3
(18) M/40	t-M6‡	CTX	8·3	3	2	0·15	25	m-Amsa, Ara-C, BMT/CR	2
(19) F/64	t-MDS→M6	MOPP	9·6	1·6	14	<0·1	8	HD-Ara-C/NR	1
(20) F/68	t-MDS→M6	HU	9·3	4·2	10	0·1	57	LD-Ara-C/NR	3

Abbreviations: CR: complete remission, defined by normal G/E ratio and less than 5% BM blasts, NR: no response; bls: blasts; E bls: erythroblasts; PB: peripheral blood; NE: anamnestically not exposed; t-M6: therapy-related AML-M6; HD-Ara-C: high-dose cytarabine; LD-Ara-C: low-dose cytarabine; DNR: daunorubicin; HU: hydroxyurea; 6-MP: 6-mercaptopurine; VP-16: etoposide; m-Amsa: m-Amsacrine; MPH: melphalan; CTX: cyclophosphamide; MOPP: mechloretamine, vincristine, procarbazine, prednisone; PCZ: procarbazine; BMT: allogeneic bone marrow transplant; NA: not available.

*From diagnosis, +indicates the patient is still alive.

†Radiographic contrast medium (see Sadamori *et al*, 1987).

‡MDS with major erythroid component according to the FAB classification.

therapy was given to 15 patients, of whom six attained complete remission. Haematologic data, outcome of remission, induction and survival are reported in Table I.

(b) *Cytology*

PAS positivity of erythroblasts was observed in 10 out of 15 patients (Table II). Two sub-groups of patients emerged from morphologic analysis of the erythroid lineage in this series. The first was characterized by partial maturation arrest with accumulation of early erythroid precursors, whereas in the second, maturation appeared to progress to the later stages of differentiation (Fig 1). Fourteen patients (nos. 1–11, 16–18), all showing leftward shift of erythropoiesis, belonged to the first sub-group in which abnormal proerythroblasts and basophilic erythroblasts represented, as a rule, more than 50% of erythroid cells (Table II). In some patients (nos. 1, 3, 6, 7) the majority of immature erythroid cells was markedly abnormal nucleo-cytoplasmic asynchrony in part of the cells made their assignment to any physiologic stage of differentiation (Castoldi & Beutler, 1988) difficult. In the second sub-group, including six patients (nos. 12–15, 19, 20), only minimal increase of early erythroblasts was observed and the sum of proerythroblasts and of basophilic erythroblasts never exceeded 25% of all erythroid cells. Maturation to the later stages of erythroid differentiation, though morphologically abnormal, was quantitatively preserved.

Classification of blast cells according to the FAB criteria, not counting erythroblasts, showed myeloid differentiation (M1 or M2) in seven cases and myelomonocytic features (M4) in one case. In eight cases blast cells were undifferentiated. Slides were not available in four patients.

Various degrees of myelodysplasia involving multiple cell lineages were documented in initial BM smears in all patients. According to the criteria proposed by Brito-Babapulle *et al* (1987), 12 of 18 patients had AML with TMDS.

(c) *Immunological studies*

Because distinction between immature erythroblasts and blast cells under phase-contrast microscopy is not clear-cut, the percentage of positive cells (see Table II) in each patient is referred to the total cellularity of the mononuclear layer. For this reason, a low cut-off point (5%) for monoclonal antibody positivity was chosen. In order to facilitate interpretation of results, percentages of erythroblasts and of blast cells in the

Table II. Cytological features and immunophenotype of the 20 patients

Patient	E1+E2/ E3+E4 (% ANC)	PAS positive E bls	BM* dysplasia	% E bls/ % bls†	Immunophenotype ‡
1	38/29	Yes	TMDS	61/32	GPA+++,CD34+,CD33++,CD15−,CD41+/−,CD7−,CD10−,Tdt−
2	48/32	Yes	TMDS	75/81	GPA+++,CD34+,CD13+CD15+,CD41−,CD10−
3	28/25	ND	E,G	50/31	GPA+++,CD34+,CD33−,CD13−,CD14−,CD41−
4	30/31	ND	E,MK	59/25	CD34+,CD13++,CD14+,CD15+,CD41−,CD10−
5	38/34	Yes	TMDS	70/12	GPA+++,CD34−/+,CD33+,CD13++,CD14−,CD41+/−,CD10−,CD7−
6	32/21	Yes	TMDS	51/14	GPA+++,CD34+/−,CD33+,CD13+/−,CD14−,CD41+/−,Tdt−
7	40/15	Yes	TMDS	41/30	GPA+++,CD34++,CD14−,CD41−,CD10−,CD7−
8	41/36	Yes	TMDS	71/26	CD34+,CD33++,CD13+,CD14+/−,CD41−,CD10−,CD7−
9	31/27	No	TMDS	NA	CD34+,CD15+
10	31/34	No	TMDS	NA	CD13+,CD15−,CD2−
11	39/22	ND	E,G,MK	81/15	GPA+++,CD34+,CD33+,CD14−,CD41+,CD10−,CD7−
12	11/41	ND	TMDS	40/38	GPA+++,CD34++,CD33+++,GD13+++,CD15++,CD14+,CD41++, CD19+,CD7+,Tdt−
13	15/65	No	E,G	46/34	GPA+++,CD34+,CD13+/−,CD15−,CD41+/−,CD19−,CD7−
14	11/45	Yes	E,MK	NA	GPA++,CD33+,CD41−,CD7+,Tdt+
15	13/39	Yes	E,G	71/20	CD34−,CD33+,CD13+,CD41+,CD10−,CD19−,CD7−
16	32/28	ND	TMDS	NA	CD34++,CD33−,CD13+,CD15+,CD41−
17	36/29	Yes	E,G,MK	NA	CD15+,CD2−
18	43/34	Yes	E,G,MK	60/18	GPA+++,CD34+,CD33++,CD13+,CD14+,CD41+,CD10−,CD7−
19	15/45	No	TMDS	55/30	CD34++,CD15+/−,Tdt−
20	16/49	No	TMDS	58/29	GPA+++,CD34−,CD33+,CD13+,CD2−,CD10−

*TMDS denotes *de novo* AML with trilineage myelodysplasia; E denotes dyserythropoiesis, G dysgranulopoiesis, MK dysmegakaryocytopoiesis.
†Cells of the mononuclear layer for immunologic analysis (100 cells observed on cytospin preparation stained with May-Grünwald-Giemsa).
‡+/−: 5–10%; +: 10–20%; ++: 20–50%; +++: >50% positive cells.
Other abbreviations: E1+E2: proerythroblasts+basophilic erythroblasts; E3+E4: polycromatophilic and orthocromatic erythroblasts; ANC: all nucleated BM cells; GPA: glycophorin A; ND: not done; PAS: Periodic acid-Schiff.

mononuclear layer are reported along with the immunophenotype in Table II.

Monoclonal antibody anti-GPA was positive in all patients tested and in four cases (nos. 3, 6, 7, 13) the percentage of positive cells was greater than the percentage of erythroblasts morphologically recognizable in the mononuclear layer. The expression of myeloid associated antigens such as CD33 and CD13 was detected in most patients and corresponded fairly well with the presence of blast cells (Table II). Cells expressing the CD41 platelet marker were detected in eight of 15 patients. Monoclonal antibody My10 (CD34) was positive in all cases tested, except in patients 15 and 20.

(d) *Cytogenetics*
Results of chromosome investigations are detailed in Table III. Karyotype at leukaemia presentation was abnormal in 18 patients. MAKA occurred in 14 patients with *de novo* EL (nos. 1–11) and therapy-related EL (nos. 16–18) in which three or more events of translocation or non-disjunction were present. This cytogenetic group corresponded to the sub-group defined on a cytological basis by the presence of left shift of erythropoiesis.

Normal karyotypes and MIKA were found in six cases (nos. 12–15, 19, 20) and were associated with the sub-group of patients showing preserved erythroid maturation.

Monosomy or long arm deletions of chromosome 5 and/or 7 occurred frequently (15/18 cases) in patients with MIKA and MAKA.

Breaks involving the short arm of chromosome 16 or chromosome 11 at the band p15, on which globin genes are located (Morton *et al*, 1984; Nicholls *et al*, 1987) were found in five cases.

DISCUSSION

(a) *Multilineage involvement*
The first part of the discussion will focus on the evidence for multiple-cell-lineage involvement in our patients. Combined morphologic, immunologic and cytogenetic studies represent a valuable tool providing indirect evidence of the occurrence of multilineage differentiation of the malignant clone in myeloid neoplasias (Third, MIC, 1988; Cuneo *et al*, 1989).

Morphologic studies in this series showed various degrees of myelodysplasia in all patients, 12 of which fulfilled the criteria for the diagnosis of AML with TMDS. The presence of myelodysplastic features in AML identifies a disease entity in which the involvement of early stem cells results in leukaemia evolving through a phase of trilineage dysmyelopoiesis, as demonstrated also by myelodysplastic relapses of some patients (Brito-Babapulle *et al*, 1988). Thus the presence of such cytologic features in our patients, besides a myelodysplastic phase clinically apparent in some of them, indicates

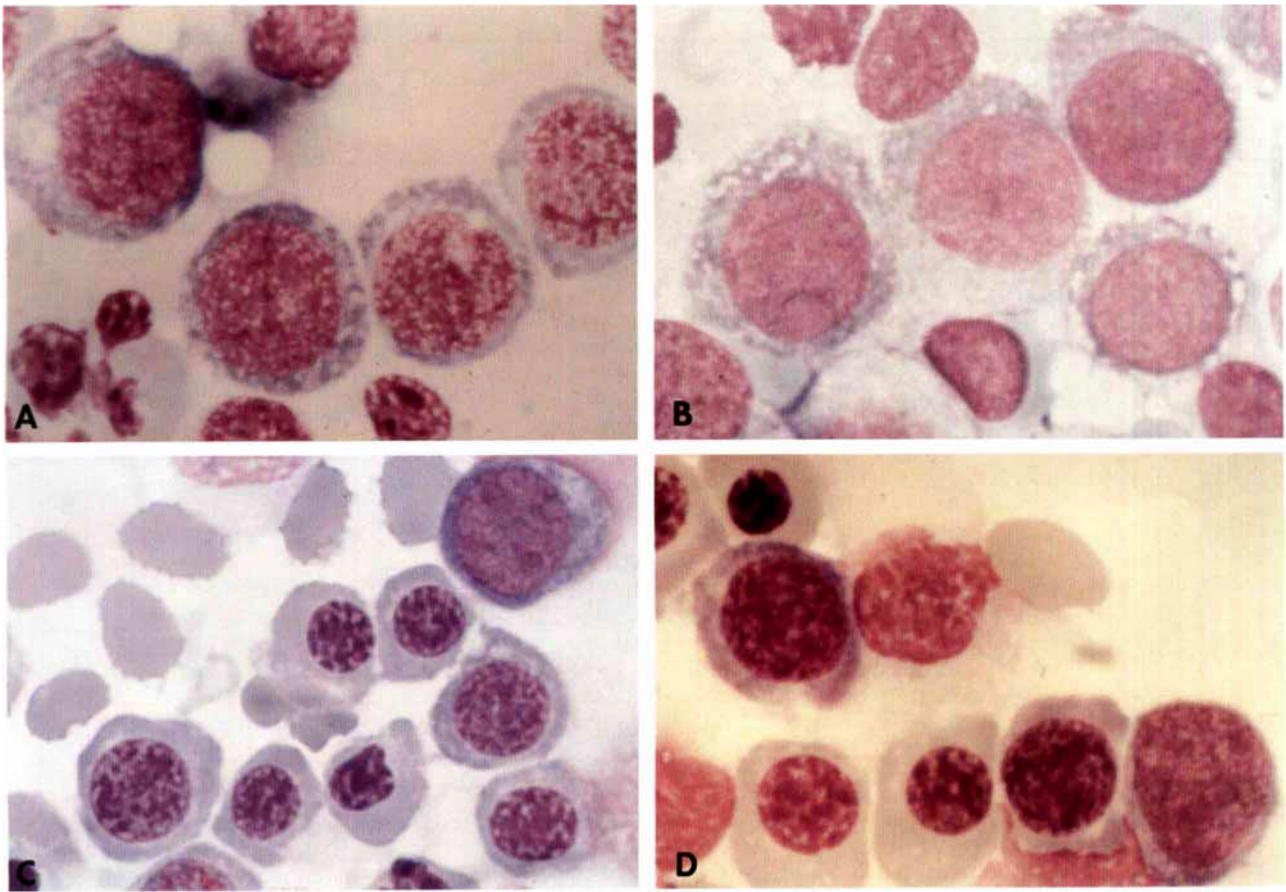

Fig 1. Microphotographs show the two cytological types of EL. (A, B) EL cells with maturation arrest; (C, D) erythroblasts with preserved maturation.

that, based on morphologic groups, multilineage involvement does occur, as a rule in EL.

Immunologic findings in our patients strengthen this view in that they show the presence of a sub-population of cells expressing platelet markers in more than 50% of cases tested as well as the presence of a minor myelomonocytic component, confirming data previously reported in early erythroblastic leukaemia (Villeval *et al*, 1986). The presence of glycophorin A positive cells exceeding in some cases the number of erythroblasts present in the Ficoll mononuclear layer suggests that some of the blast cells may express this erythroid associated antigen (Greaves *et al*, 1983). The proportion of cases expressing the CD34 surface marker is high as compared with previously reported series including AML patients (Vaughan *et al*, 1988; Borowitz *et al*, 1989). Although the percentage of CD34 positive cells is low in most of our cases (see Table II), it should be emphasized that a good correspondence was observed between the number of blast cells in the mononuclear layer and the percentage of cells labelled by the My10 monoclonal antibody. Double labelling experiments are warranted in future investigations to assess whether CD34 positive cells in EL coexpress later differentiation markers such as CD15, as the immunophenotype of some of our patients seems to suggest (see Table II), or whether they may represent a subset of blast cells with a very immature phenotype (Katz *et al*, 1985).

Cytogenetically, the frequent occurrence of −5/5q− anomaly, often associated with −7/7q− in the context of complex karyotypes, indicates the existence of striking similarities with cytogenetic patterns of therapy-related leukaemias (Whang-Peng *et al*, 1988), which typically represent myeloid stem cell disorders (McKenna *et al*, 1981). Also, aberrations exhibited by patients with MIKA, such as t(1;3)(p36; q21), del(20)(q11) and +11, having been reported in a spectrum of myeloid neoplasias (Rege-Cambrin *et al*, 1986; Davis *et al*, 1984; Takasaki *et al*, 1988) are consistent with the involvement of undifferentiated stem cells retaining, potentially, the capability to differentiate along multilineage pathways.

Prospective studies, employing newly developed techniques (Keinanen *et al*, 1988; Berneman *et al*, 1988), will hopefully provide a direct demonstration of the occurrence of multilineage involvement in EL.

In this series the overlap existing between *de novo* EL and therapy-relatd EL in terms of clinical features, of morphologic, immunologic and cytogenetic aspects is striking. Indeed pancytopenia, myelodysplasia, anomalies of chromosomes 5 and 7 and the involvement of early stem cells are

Table III. Cytogenetic findings of the patients studied

Patient diagnosis (FAB)	Karyotype	Abnormal cells/ normal cells	Cytogenetic group*	Maturation arrest of E bls
(1) M6	45,XX,t(4;?)(q33;?),del(5)(q14q31), inv (3) (q21q35),del(12)(q23),del(15)(q23), −16, −17, +M	10/0	Typical MAKA	Yes
(2) M6	46,XV, + 1, − 5,t(11;?)(p15;?), −16, +der(16)t(1;16)(p11;p11), −18, +M	12/0	Typical MAKA	Yes
(3) M6	46,XY,dup(1)(q24→qter),del(8)(p12), t(9;?) (q34;?),del(13)(q14q22),t(16;?)(p11;?)	10/0	Typical MAKA	Yes
(4) M6	45,XY,t(3;?)(p25;?),del(5)(q14q31), −7, + 8,t(15;?)(q25;?), −22	15/0	MAKA	Yes
(5) M6	50,XY,t(1;?)(q12;?),del(7)(q21q31), −11,t(16;?)(p11;?)+ring, +M1, +M2, +M3, +M4	10/0	Typical MAKA	Yes
(6) M6	45,X, −Y, −5, −16, −21, +M1, +M2, +M3	15/1	Typical MAKA	Yes
(7) M6	44,XY, −5,i(17q), −20	8/2	MAKA	Yes
(8) MDS→M6	45,XX,del(5)(q12q32), −7, −9, t(13;?)(p11;?),t(14;?)(p11;?) t(16;?)(p12;?), −19, −22, +M1, +M2, +M3	10/0	Typical MAKA	Yes
(9) MDS→M6	45,X, −Y,t(1;4)(p31;q13), −3, −5, −7,t(9;?)(q12;?),del(12)(p12), t(12;?)(q24;?), −10, −17, −18, +M1, +M2, +M3 +, +M4, +M5, +M6	12/1	Typical MAKA	Yes
(10) MDS→M6	45,X, −X,del(5)(q12q32),t(9;11)(q11;p12), +15, −20	15/1	MAKA	Yes
(11) M6†	44,XY,del(1)(p35),t(2;17)(p11;q11), t(5;?)(q13;?), −12, +der(12)t(3;12)(q21;q12), −17, −19,t(20;?)(q11;?)	13/1	Typical MAKA	Yes
(12) M6	46,XY	0/20	Normal	No
(13) M6	46,XX	0/15	Normal	No
(14) MDS→M6	45,XY, −7	13/2	MIKA	No
(15) MDS→N6	47,XY, + 11,del(20)(q11)	12/6	MIKA	No
(16) t-M6	45,XY, −5, −19, −20, +M1, +M2	10/10	Typical MAKA	Yes
(17) t-MDS→M6	45,XY, +Y, −5, −7, −18,t(19;?) (?q;?),t(19;?)(?q;?), +M1	15/0	Typical MAKA	Yes
(18) t-M6†	47,XY,t(1;?)(q44;?),t(2;?) (p25;?),t(3;6)(p11;p24); −7, −9 t(9;?)(p23;?), −17,t(19;?)(q11;?), −21, + 22, +M1, +M2, +M3, +M4	8/12	Typical MAKA	Yes
(19) t-MDS→M6	46,XY,t(1;3)(p36;q21)	15/0	MIKA	No
(20) t-MDS→M6	46,XX,del(7)(q31),t(18;?) (p11;?)	10/0	MIKA	No

*MAKA is defined by at least three events of translocation or non-disjunction. MIKA by one or two such events. 'Typical MAKA' are MAKA patients in which additional features such as karyotype instability, markers or ring chromosomes, a mode of 47 chromosomes or less are present (Sandberg, 1980).

†MDS with major erythroid component according to the FAB classification.

typical features of secondary blood neoplasias and their presence in *de novo* EL provides compelling, though indirect evidence of toxic aetiology. Exposure to potentially carcinogenic compounds could be documented in a proportion of cases (7/15) which seems to be higher than in other series including AML patients (Mitelman *et al*, 1981). As previously outlined, retrospective studies are unlikely to allow for reliable assessment of exposure to mutagens in all cases (Lawler *et al*, 1979). Prospective analyses are in progress and will hopefully define the relationship between environmental exposure and leukaemogenesis more exactly (Farrow *et al*, 1989).

(b) *Two cytogenetic–clinicopathological types of EL*

The diagnosis of EL according to the FAB criteria requires the presence of more than 50% erythroblasts in the BM, irrespective of their morphologic features (Bloomfield & Brunning, 1985). Indirect evidence has been provided in this paper that EL is a stem cell disorder evolving through a myelodysplastic phase; hence it is not surprising that heterogeneous patterns of erythroid involvement may occur in the evolutive phases of this neoplastic disorder.

Two cytological pictures, the first with pathologic increase of early erythroblasts and the second with preserved maturation of erythroid cells, were observed in this series in 14 and

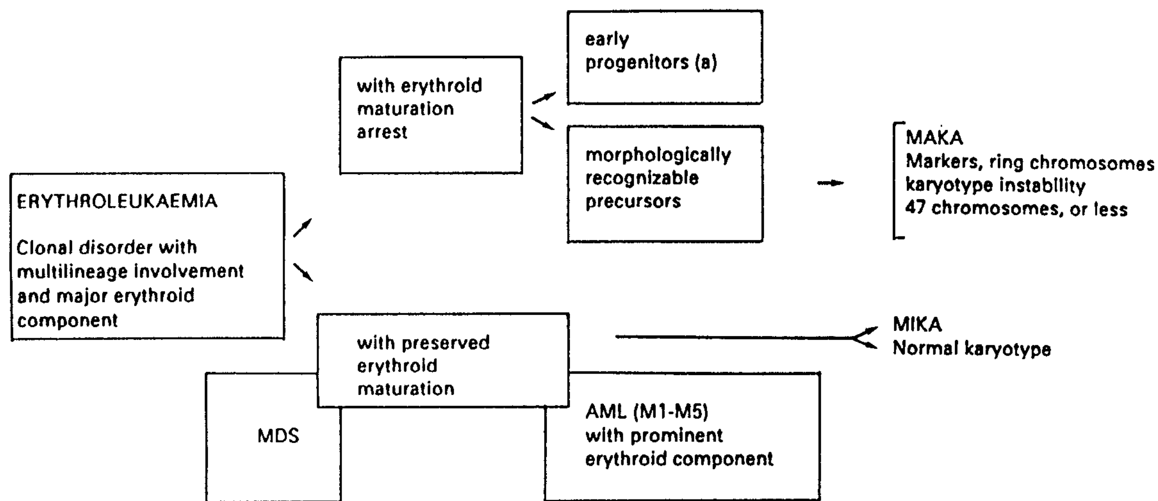

Fig 2. Schematic representation of the nosography of erythroleukaemia and its correlation with cytogenetic patterns. EL with preserved erythroid maturation shows partial overlap with MDS and AML with prominent dyserythropoiesis. (a) Disease entity recognized by means of ultrastructural and immunological features. In some cases complex karyotypes are reported (Villeval *et al*, 1986).

six cases, respectively. We have shown that MAKA are associated with the former group, whereas MIKA or normal karyotypes appear to be recurrent in the latter.

Although MAKA were always observed in this report in EL with maturation arrest of erythroid cells, this association is unlikely to be absolute and, indeed, complex karyotypes have also been found in MDS and AML, possibly reflecting, in some cases, environmental exposure to myelotoxic agents (Yunis *et al*, 1988).

In search of confirmation of our findings we reviewed the literature and found 16 cases of AML-M6 in which the existence of this cytologic–cytogenetic association could be evaluated. Bernheim *et al* (1983) reported five patients with excess of proerythroblasts and of basophilic erythroblasts, three of which had MAKA, one had MIKA, and one had normal karyotype. The last two patients belonged to the pediatric age group in which pathogenesis and cytogenetics of leukaemia is believed to be different from that in adult patients (Brodeur *et al*, 1983; Stamberg, 1987). In the same series two patients had MAKA without leftward shift of erythropoiesis; one of the two, however, had congenital Fanconi's anaemia as a possible predisposing factor. The remaining nine adult patients had normal karyotypes and preserved erythroid maturation. Thus this association also appears to be quite strong in previously reported adult patients.

Seemingly, gross disruption of mitotic mechanisms may give rise to the extreme degree of pathologic evolution of the neoplastic clone, a process mirrored at the cytological level by the inability of erythroblasts to mature beyond a given stage of differentiation. In some cases complex karyotypes may show alterations of chromosome regions such as 11p or 16p possibly related to erythroid differentiation (Kirsch *et al*, 1985).

These two newly identified cytogenetic-cytopathological types of EL seem to exhibit clear differences.

Cytologically, early erythroblasts were, as a rule more than 50% of all erythroid cells in the group with maturation arrest and never exceeded 25% in the group with preserved maturation.

MIKA and MAKA differed, on cytogenetic grounds, more than one would predict on the basis of the criteria chosen for their distinction, namely the occurrence of two or three aberrant events. Indeed, the majority of patients with three or more events of translocation or non-disjunction (see Table III), here referred to as MAKA, presented additional features such as karyotype instability, markers or ring chromosomes, a mode of 47 chromosomes or less, thus representing 'typical MAKA' as previously defined (Sandberg, 1980).

Although the cases reported here are few in number and caution is required before drawing firm conclusions, some clinical findings appear to be different in the two cytogenetic-clinicopathological groups. Patients with maturation arrest of erythroblasts are associated with lower haemoglobin levels (range 4·4–10·8 g/dl, median 7·4) as compared to the group with preserved erythroid maturation (range 8·1–10·3 g/dl, median 9·45). Three of 12 patients in the maturation arrest-MAKA group attained complete remission after chemotherapy, versus three out of five patients in the preserved maturation-MIKA/normal karyotype group. Survival ranged between 1 and 10+ months, median 3·5 months, in the former group and between 1 and 17+ months, median 13 months, in the latter. These data are consistent with previous cytogenetic reports, where MAKA were shown to correlate with shorter survival (Sandberg, 1980; Nakamura, 1989). Thus, patients with maturation arrest-MAKA appear to represent a sub-group with poor prognosis.

In conclusion, morphologic–immunologic–cytogenetic studies suggest that EL, as defined by the FAB group, is a multilineage proliferation presenting a spectrum of clinico-biological features from which two cytogenetic–cytopathological types can be delineated (Fig 2).

Leukaemias of erythroid lineage have recently been shown to extend themselves beyond the morphologic boundaries of the FAB classification, since immunologic and ultrastructural studies disclosed leukaemic forms with very early erythroid features (Breton-Gorius *et al*, 1987). These findings point to the need to focus attention on erythroid blast cells in the cytologic classification of erythroleukaemia. The two cases reported here (nos. 11, 18) with MDS with major erythroid component and left-shifted erythropoiesis, for which a clinical diagnosis of EL was felt to be more appropriate, raise the same question concerning the role of morphologically recognizable early erythroblasts in the diagnosis of erythroleukaemia. Clarification of these points appears to be necessary to cover the whole area of erythroid leukaemias with a reproducible cytologic classification of this variegated neoplastic disorder.

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REFERENCES

Bennett, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A.G., Gralnick, H.R. & Sultan, C. (1985) Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French–American–British Cooperative Group. *Annals of Internal Medicine*, **103**, 626–629.

Berneman, Z.N., van Bockstaele, D.R., Van den Bergh, M., Van Roy, B., Roete, L., De Bock, R., Korthout, M., Peetermans, M.E. & Dumon, J. (1988) Karyotypic evidence for the leukemic involvement of the erythroblasts in erythroleukemia. *Leukemia*, **2**, 296–299.

Bernheim, A., Berger, R., Daniel, M.T., Valensi, F. & Flandrin, G. (1983) Malignant and reactive erythroblasts in erythroleukemia (M6). *Cancer Genetics and Cytogenetics*, **10**, 1–10.

Bloomfield, C.D. & Brunning, R.D. (1985) The revised French–American–British classification of acute myeloid leukemia: is new better? *Annals of Internal Medicine*, **103**, 614–616.

Borowitz, M.J., Gockerman, J.P., Moore, J.O., Civin, C.I., Page, S.O., Robertson, J. & Bigner, S.H. (1989) Clinicopathologic and cytogenetic features of CD34 (My10)-positive acute nonlymphocytic leukemia. *American Journal of Clinical Pathology*, **91**, 265–170.

Breton-Gorius, J., Villeval, J.L., Mitjavila, M.T., Vinci, G., Guichard, J., Rochant, H., Flandrin, G. & Vainchenker, W. (1987) Ultrastructural and cytochemical characterization of blasts from early erythroblastic leukemias. *Leukemia*, **1**, 173–181.

Brito-Babapulle, F., Catovsky, D. & Galton, D.A.G. (1987) Clinical and laboratory features of *de novo* acute meyloid leukaemia with trilineage myelodysplasia. *British Journal of Haematology*, **66**, 445–450.

Brito-Babapulle, F., Catovsky, D. & Galton, D.A.G. (1988) Myelodysplastic relapse of *de novo* acute myeloid leukaemia with trilineage myelodysplasia: a previously unrecognized correlation. *British Journal of Haematology*, **68**, 411–415.

Brodeur, G.M., Williams, D.L., Kalwinsky, D.K., Williams, K.J. & Dahl, G.V. (1983) Cytogenetic features of acute nonlymphocytic leukemia in 73 children and adolescents. *Cancer Genetics and Cytogenetics*, **8**, 93–105.

Castoldi, G.L. & Beutler, E. (1988) Erythrocytes. In: *Atlas of Blood Cells. Function and Pathology* (ed. by D., Zucker-Franklin, M. F., Greaves, C. E. Grossi and A. M. Marmont). Lea and Febiger, Philadelphia.

Castoldi, G.L., Cuneo, A. & Tomasi, P. (1989) Phenotype-related chromosome aberrations and stem cell involvement in acute myeloid leukemia. *Haematologica*, **74**, 911–916.

Cuneo, A., Mecucci, C., Kerim, S., Vandenberghe, E., Dal Cin, P., Van Horsoven, A., Rodhain, J., Bosly, A., Michaux, J.L., Martiat, P., Boogaerts, M., Carli, M.G., Castoldi, G.L. & Van Den Berghe, H. (1989) Multipotent stem cell involvement in megakaryoblastic leukemia: cytological and cytogenetic evidence in 15 patients. *Blood*, **74**, 1781–1790.

Dameshek, W. (1969) The DiGuglielmo syndrome revisited. *Blood*, **34**, 567–572.

Davis, M.P., Dewald, G.W., Pierre, R.V. & Hoagland, H.C. (1984) Hematologic manifestation associated with deletions of the long arm of chromosome 20. *Cancer Genetics and Cytogenetics*, **12**, 63–71.

Farrow, A., Jacobs, A. & West, R.R. (1989) Myelodysplasia, chemical exposure, and other environmental factors. *Leukemia*, **3**, 33–35.

Freedman, M.H., Chan, H.S.L. & Chang, S.L. (1986) Childhood erythroleukemia. Studies on pathogenesis using colony assays. *American Journal of Pediatric Hematology/Oncology*, **8**, 2–7.

Greaves, M.F., Sieff, C. & Edwards, P.A.W. (1983) Monoclonal antiglycophorin as a probe for erythroleukemias. *Blood*, **61**, 645–651.

Griesser, G.H. & Horny, H.P. (1987) Hemopathological features of acute erythremia (morbus Di Guglielmo). Contribution to the classification and differential diagnosis of erythroid neoplasias. *Acta Haematologica*, **77**, 193–197.

Griffin, J.D., Davis, R., Nelson, D.A., Davey, F.R., Mayer, R.J., Schiffer, C., McIntyre, C.R. & Bloomfield, C.D. (1986) Use of surface marker analysis to predict outcome of adult acute myeloblastic leukemia. *Blood*, **68**, 1232–1241.

Hetzel, P. & Gee, T.S. (1978) A new observation in the clinical spectrums of erythroleukemia: a report of 46 cases. *American Journal of Medicine*, **64**, 765–777.

Inoue, S., Ravindranath, Y. & Zueller, W.W. (1975) Cytogenetic analysis of erythroleukemia in two children: evidence of nonmalignant nature of erythron. *Scandinavian Journal of Haematology*, **14**, 129–139.

Katz, F.E., Tindle, R., Sutherlands, D.R. & Greaves, M.F. (1985) Identification of a membrane glycoprotein associated with haemopoietic progenitor cells. *Leukemia Research*, **9**, 191–198.

Keating, M.J., Cork, A., Broach, Y., Smith, T., Walters, R.S., McCredie, K.B., Trujillo, J. & Freireich, E.J. (1987) Toward a clinically relevant cytogenetic classification of acute myelogenous leukemia. *Leukemia Research*, **11**, 119–133.

Keinanen, M., Griffin, J.D., Bloomfield, C.D., Machnicki, J. & de la Chapelle, A. (1988) Clonal chromosomal abnormalities showing multiple-cell-lineage involvement in acute myeloid leukemia. *New England Journal of Medicine*, **318**, 1153–1158.

Kirsch, I.R., Brown, J.A., Lawrence, J., Korsmeyer, S.J. & Morton, C.C. (1985) Translocations that highlight chromosomal regions of a differentiated activity. *Cancer Genetics and Cytogenetics*, **18**, 159–171.

Lawler, S.D., Summersgill, B.M., Clinck, H.McD. & McElwain, T.J. (1979) Chromosomes, leukemia and occupational exposure to leukemogenic agents. *Lancet*, **i**, 853–654.

McKenna, R., Parkin, J.L., Foucar, K. & Brunning, R.D. (1981) Ultrastructural characteristics of therapy-related acute nonlymphocytic leukemia: evidence for a panmyelosis. *Cancer*, **48**, 725–737.

Mitelman, F., Brandt, L., Alimena, G. & Dallapiccola, B. (1981) Chromosome pattern, occupation, clinical features in patients with acute nonlymphocytic leukemia. *Cancer Genetics and Cytogenetics*, **4**, 197–214.

Morton, C.C., Kirsch, I.R., Taub, R., Orkin, S.H. & Brown, J.A. (1984) Localization of the beta-globin gene by chromosomal in situ hybridization. *American Journal of Human Genetics*, **36**, 576–585.

Nakamura, H. (1989) Cytogenetic heterogeneity in erythroleukemia defined as M6 by the French–American–British (FAB) Cooperative Group criteria. *Leukemia*, **3**, 305–309.

Nicholls, R.D., Jonasson, J.D., McGee, J.O.D., Patil, S., Ionasescu, W., Weatherall, D.J. & Higgs, D.R. (1987) High resolution gene mapping of the human alpha-globin locus. *Journal of Medical Genetics*, **24**, 39–46.

Rege-Cambrin, G., Mecucci, C., Van Orshoven, A., Tricot, G. & Van Den Berghe, H. (1986) Translocation t(1;3) (p36;q21) in malignant myeloid stem cell disorders. *Cancer Genetics and Cytogenetics*, **22**, 75–84.

Roggli, V.L. & Saleen, A. (1982) Erythroleukemia: a study of 15 cases and literature review. *Cancer*, **49**, 101–108.

Sadamori, N., Miyajima, J., Okajima, S., Shima, M., Yano, M., Koga, M., Kawamoto, M., Fujii, H., Mori, T. & Ichimaru, M. (1987) Japanese patients with leukemia following the use of Thorostrast including a patient with marked chromosomal rearrangements. *Acta Haematologica*, **77**, 11–14.

Sandberg, A.A. (1980) *The Chromosomes of Human Cancer and Leukemia.* Elsevier North-Holland, New York.

Second MIC Co-operative Study Group (1988) Morphologic, immunologic cytogenetic (MIC) working classification of acute myeloid leukemias. *Cancer Genetics and Cytogenetics*, **30**, 1–15.

Stamberg, J. (1987) Chromosome abnormalities in erythroleukemia. *Cancer*, **60**, 2649–2653.

Suda, T., Sato, Y., Furukawa, Y., Suda, J., Eguchi, M., Saito, M. & Miura, Y. (1986) Myeloid and erythroid lineage expression of haemopoietic progenitors derived from an abnormal clone in erythroleukaemia. *British Journal of Haematology*, **64**, 647–656.

Takasaki, N., Kaneko, Y., Maseki, N. & Sakurai, M. (1988) Trisomy 11 in chronic myelomonocytic leukemia: report of two cases and review of the literature. *Cancer Genetics and Cytogenetics*, **30**, 109–117.

Third MIC Co-operative Study Group (1988) Morphologic, immunologic and cytogenetic (MIC) working classification of the primary myelodysplastic syndromes and therapy-related myelodysplasias and leukemias. *Cancer Genetics and Cytogenetics*, **32**, 1–10.

Vaughan, W.P., Civin, C.I., Weisenburger, D.D., Karp, J.E., Graham, M.L., Sanger, W.G., Grierson, H.L., Joshi, S.S. & Burke, P.J. (1988) Acute leukemia expressing the normal human hematopoietic stem cell membrane glycoprotein CD34 (My10). *Leukemia*, **2**, 661–666.

Villeval, J.L., Cramer, P., Lemoine, F., Bettaieb, A., Bernaudin, F., Berger, R., Flandrin, G., Breton-Gorius, J. & Vainchenker, W. (1986) Phenotype of early erythroblastic leukemias. *Blood*, **68**, 1167–1174.

Whang-Peng, J., Young, R.C., Lee, E.C., Longo, D.L., Schechter, G.P. & De Vita, V.T., Jr (1988) Cytogenetic studies in patients with secondary leukemia/dysmelopoietic syndrome after different treatment modalities. *Blood*, **71**, 403–414.

Yunis, J.J., Lobell, M., Arnesen, M.A., Oken, M.M., Mayer, M.G., Rydell, R.E. & Brunning, R.D. (1988) Refined chromosome study helps define prognostic subgroups in most patients with primary myelodysplastic syndrome and acute myelogenous leukaemia. *British Journal of Haematology*, **68**, 189–194.