Biphenotypic leukaemia: a case of mixed T lymphoblastic and myeloblastic leukaemia

MAM MORGAN, CS SCOTT, J TAVARES DE CASTRO,* HJ LIMBERT, N POLLI,*
D CATOVSKY,* RD PYRAH,† BE ROBERTS

From the Department of Haematology, Regional Radiotherapy Centre, Cookridge Hospital, Leeds, the *MRC Leukaemia Unit, Royal Postgraduate Medical School, London, and †Airedale General Hospital, Keighley, W Yorkshire

SUMMARY A case of mixed acute leukaemia with T lymphoblastic, myeloblastic, and monocytic components is described. The use of immunological markers, ultrastructural morphology, cytochemistry, immunochemistry, and combined techniques, simultaneously detecting two markers in individual cells, made it possible to define the different blast cell populations.

Neoplastic disorders are believed to arise from the uncontrolled clonal proliferation of a single progenitor cell. With the use of monoclonal antibody techniques, an increasing number of leukaemias and lymphomas¹⁻³ are found to have a mixed phenotype. A biphenotypic leukaemia is one in which leukaemic cell populations with apparent myeloid and lymphoid characteristics coexist. This is in contrast to leukaemias of hybrid phenotype, in which leukaemic cells show phenotypic features of more than one cell line, suggesting the existence of lineage infidelity.⁴ This report describes a case of mixed leukaemia characterised by two distinct populations of blasts: one showed the features of acute T lymphoblastic leukaemia, the other acute myeloblastic leukaemia with some monocytic differentiation. The biphenotypic nature of this leukaemia was established by light microscopy, morphology and cytochemistry, monoclonal antibody studies, and electron microscopy.

Case report

A 65 year old woman presented with an acute leukaemia. She had a history of general malaise, recurrent infections, mouth ulcers, easy bruising, and weight loss. The salient clinical features were: fever 38°C, anaemia, bruising on the limbs, 2 cm × 1 cm tender inguinal node, palpable spleen tip, and 6 cm hepatomegaly. There was no mediastinal mass on a chest radiograph. A full blood count

showed haemoglobin 6.0 g/dl, white cell count $167 \times 10^9/l$ (differential 98% blasts, 2% lymphocytes, with an occasional granulocyte) and platelet count $65 \times 10^9/l$. The biochemical profile was normal and *Escherichia coli* was isolated from a urine culture. A bone marrow aspirate was hypercellular with sheets of blasts, the morphological and immunological features of which are described below.

She received induction chemotherapy with daunorubicin, vincristine, and prednisolone and a bone marrow aspirate performed eight weeks later indicated complete remission. Maintenance chemotherapy was started comprising methotrexate, 6-mercaptopurine, cytosine arabinoside, vincristine, and prednisolone. The patient remains in first remission on maintenance chemotherapy 16 months after presentation.

MATERIAL AND METHODS

Light microscopy, morphology, and cytochemistry

The morphology of the peripheral blood and bone marrow was examined on May-Grunwald-Giemsa stained smears. Cytochemical staining of blood and bone marrow for myeloperoxidase, Sudan black, chloroacetate esterase, α -naphthyl acetate esterase, periodic acid Schiff, and acid phosphatase were performed by standard techniques.

Immunological phenotyping

Peripheral blood whole leucocyte and mononuclear cell fractions were separated by Plasmagel induced red blood cell rouleaux formation and Lymphoprep density gradient centrifugation respectively. Both fractions comprised in excess of 98% blasts. Immunological phenotyping was performed by (a)

detection of cell surface antigens by indirect immunofluorescence of living cell suspensions using a panel of monoclonal antibodies (specificities, sources, and references listed in Table 1); (b) demonstration of terminal deoxynucleotidyl transferase (TdT) with a rabbit anticalf TdT serum by indirect immunofluorescence of methanol fixed cytocentrifuge preparations⁵; and (c) E rosetting with sheep red blood cells.

To assess the expression of two markers in the same cell, combinations of TdT with E rosettes, 3A1, and My9 were performed as follows: (a) E rosette preparations were cytocentrifuged, fixed, and processed for TdT as above; (b) 3A1 and My9 were detected in cell suspension using a fluorescein conjugated secondary antibody, cytocentrifuged cell monolayers prepared, and TdT examined using a rhodamine conjugated secondary antibody. Negative controls were obtained by omitting the primary antibody or by using an irrelevant antibody.

Electron microscopy

Cells were processed for ultrastructural morphology and detection of myeloperoxidase at electron microscopy level using conventional techniques.⁶ The demonstration of cell surface antigens by monoclonal antibodies and the immunogold method

simultaneously with cytoplasmic myeloperoxidase was achieved as previously described.⁶ The monoclonal antibodies used in these combination techniques were: antigranulocyte FMC13;⁷ antimonocyte FMC17 and FMC32;^{8,9} OKM1;¹⁰ anti-T cell 3A1;¹¹ and an anti-HLADr equivalent, FMC4.¹²

RESULTS

Light microscopy

Examination of May-Grunwald-Giemsa stained blood and bone marrow smears showed two populations of blasts. About 70% of these were typical lymphoblasts with a high nuclear to cytoplasmic ratio and convoluted nucleus, while the remainder showed the features of myeloblasts, with a lower nuclear to cytoplasmic ratio, a single nucleolus, and occasional granules in the cytoplasm. Cytochemically, 60% of blasts showed weak to moderate focal acid phosphatase activity, 10% of blasts showed weak myeloperoxidase and Sudan black reactivity, and there was no significant chloroacetate esterase, α -naphthyl acetate esterase, or periodic acid Schiff activity. These preliminary results indicated two populations of blasts: a minor proportion showing myeloid differentiation with the remainder having the typical acid phosphatase focal positivity suggestive of T cell acute lymphoblastic leukaemia blasts.

Table 1 Immunological phenotype of the blast cells

Marker	% Positive cells*	Specificity	Source	Reference
Precursor cells				
TdT	69	Terminal transferase	PL/Pharmacia	5
FMC4	60	HLA-Dr	Sera Lab	12
I2	40	HLA-Dr	Coulter	28
J5	<1	Common acute lymphoblastic leukaemia antigen	Coulter	29
T cells				
OKT17	50	T cells	Dr G Goldstein	30
3A1	30	T cells	Prof B Haynes	11
OKT11	25	Sheep red blood cell receptor	Ortho	13
E-rosettes	22	Sheep red blood cell receptor		
UCHT1	5	T cells (T3 equivalent)	Dr P Beverley	31
OKT4	<1	Helper T cells	Ortho	13
OKT8	<1	Suppressor T cells	Ortho	13
OKT6	<1	Cortical thymocytes	Ortho	13
HTA-1 (NA1/34)	<1	Cortical thymocytes	Sera Lab	32
Myeloid cells				
My7	14	Granulocytes, monocytes, and precursors	Coulter	33
My9	13	Early granulocytes, monocytes, and precursors	Coulter	34
FMC17	14	Predominantly monocytes/macrophages	Dr H Zola	9
FMC32	7	Predominantly monocytes/macrophages	Dr H Zola	9
LeuM3	10	Predominantly monocytes/macrophages	Becton-Dickinson	35
My4	11	Predominantly monocytes/macrophages	Coulter	33
UCHM1	9	Predominantly monocytes/macrophages	Dr P Beverley	36
61D3	<1	Predominantly monocytes/macrophages	BRL	35
Mo2	<1	Predominantly monocytes/macrophages	Coulter	37
Other lineages				
LICR/LON/R10	<1	Glycophorin A, erythroid cells	Dr P Edwards	38
AN51	<1	Platelet glycoprotein Ib	Prof A McMichael	39
B1	<1	B cells	Coulter	40

*Blasts comprised in excess of 98% of the leucocyte fraction tested.

Fig. 1 Lymphoblast. Note the high nuclear to cytoplasmic ratio, the inconspicuous nucleolus, and two nuclear pockets (arrow). Uranyl acetate and lead citrate staining. Original magnification $\times 18\,500$.

Fig. 2 Myeloblast, myeloperoxidase reaction. Note the presence of myeloperoxidase activity in the perinuclear space (small arrow), rough endoplasmic reticulum, Golgi apparatus (asterisk), and small and medium sized granules. This pattern indicates active synthesis of the enzyme in an immature cell. A nuclear pocket (large arrow) encloses an area of cytoplasm containing myeloperoxidase reactive material. Original magnification $\times 14\,700$.

Fig. 3 Monocytoid cell, combined detection of a monocytic antigen (FMC32) and myeloperoxidase. The small myeloperoxidase containing granules and the villous cytoplasmic outline are characteristic of the monocytic lineage. The 30 nm gold particles attached to the cell membrane are signalled by arrows. Original magnification $\times 18\,400$.

Ultrastructural morphology and myeloperoxidase cytochemistry

Two populations of blasts were seen by electron microscopy: lymphoid and myeloid. Fig. 1 shows a typical lymphoblast characterised by a high nuclear to cytoplasmic ratio, slightly irregular nuclear outline, peripheral chromatin condensation with some patches of heterochromatin, and a small ring form nucleolus. Nuclear pockets were noted in a significant number of cells with both lymphoid and myeloid morphology (Figs. 1 and 2). Lymphoblasts were uniformly unreactive for myeloperoxidase. A second population of blasts, about 20% of the cells, was myeloperoxidase positive, had typical features of myeloblasts, with a lower nuclear to cytoplasmic ratio than lymphoblasts, immature nucleus with marginal chromatin condensation, and a small nucleolus. Short strands of endoplasmic reticulum and large to medium size granules were present in the cytoplasm of these cells. In the myeloblast illustrated in Fig. 2 the myeloperoxidase reaction is seen in the granules, in the perinuclear membrane, and in the endoplasmic reticulum. The nuclear membrane limiting the heterochromatin loops in the nuclear pocket contains myeloperoxidase and the cytoplasm enclosed by these loops also contains myeloperoxi-

dase reactive granules and mitochondria. Some of the myeloid cells showed monocytic features with smaller myeloperoxidase positive granules scattered to the periphery of the cytoplasm and a more villous cytoplasmic outline (Fig. 3).

Immunological studies

The results of these investigations are summarised in Table 1. The majority (69%) of blasts were TdT positive, while the expression of T lineage associated determinants indicated appreciable populations of 3A1, OKT17, and T11 positive blasts. A combined assay for membrane sheep red blood cell receptors and nuclear TdT defined three blast cell populations: (a) sheep red blood cell positive, TdT positive; (b) sheep red blood cell negative, TdT positive; and (c) sheep red blood cell negative, TdT negative. The partial expression of T11 together with the absence of detectable T4, T8, T6, and T1 (UCHT2) determinants further suggested that the T blasts were at an early stage of thymic differentiation.¹³ Studies with the myeloid associated monoclonal antibodies indicated the presence of about 13% My9/My7 (pan-myeloid) positive blasts. The results for monocyte associated monoclonal antibodies imply that most of the myeloid blasts also expressed

Table 2 Morphological, cytochemical, and immunological characteristics of the different blast cell populations

Blast type	%*	Morphology	Cytochemistry		Immunological phenotype					
			Myeloperoxidase	Acid phosphatase	TdT	E/T11	3A1/T17	My9/My7	FMC17/32	FMC13
T blasts	70	Small blasts with high nuclear to cytoplasmic ratio	—	Focal	+	—/+	—/+	—	—	—
Myeloid	30	Blasts show lower nuclear to cytoplasmic ratio; some show villous outline	Medium-large or small granules	—	—†	—	—	+	—/+‡	—/+§

*Studies performed when the peripheral blood white cell count was $167 \times 10^9/l$. Leucocyte fractions for immunological studies contained in excess of 95% blasts.

†2% of blasts coexpressed My9 and TdT.

‡Positive in monocytic cells.

§Positive in granulocytic cells.

FMC32, OKM5, UCHM1, LeuM3, and My4 but not Mo2 or 61D3 determinants. These findings support the observations of electron microscopy which suggested a monocytic component, although their lack of cytochemical α -naphthyl acetate esterase activity and absence of monocyte specific α -naphthyl acetate esterase isoenzymes (as assessed by isoelectric focusing) indicated these blasts to be at a relatively early stage of monocytic differentiation.¹⁴

Electron immunocytochemistry

Monoclonal antibody FMC13 (granulocyte associated) was reactive with the more mature granulocytic cells (myelocytes and metamyelocytes) while the majority of myeloblasts in this case were unreactive. Monoclonal antibodies FMC17 and FMC32 (monocyte associated) were reactive with about 10% of the blasts and these cells tended to have monocytoid features (Fig. 3). Monoclonal antibody OKM1 was reactive with both myeloblasts and blasts with monocytic features, although some OKM1 positive cells were myeloperoxidase negative.

3A1 positive cells invariably corresponded to blasts with lymphoid morphology. 3A1 negative cells were either myeloperoxidase positive or negative. The first subset had the morphological characteristics of myeloid cells, whereas myeloperoxidase negative cells comprised the lymphoid population. 3A1 and myeloperoxidase were mutually exclusive markers because they did not appear to coexist in any of the cell types examined.

Table 2 summarises the characteristics of the different blast cell populations in this case, defined by the integration of different techniques.

Discussion

This report describes a case of mixed T cell acute lymphoblastic and acute myeloblastic leukaemia

which was defined by extensive laboratory studies. The combination of the immunogold method with myeloperoxidase cytochemistry was particularly important in this case because (a) it confirmed the mutual exclusion in individual cells of the antigen detected by 3A1 and cytoplasmic myeloperoxidase, and (b) it was possible to dissect in the myeloid (myeloperoxidase positive) component two subpopulations of blasts—myeloblasts (FMC17/32—) and promonocytes (FMC17/32+). The identification of the 3A1 reactive cells as non-myeloid (myeloperoxidase negative) is important because 3A1 has been shown to react in some cases with a fraction of myeloid cells.¹⁵

In this case the demonstration of a T cell component was based on the presence of surface markers defined by E rosettes/OKT11, 3A1, and OKT17. The last two antibodies appear to be more sensitive in detecting early T cell differentiation than E rosettes as up to 30% of T cell acute lymphoblastic leukaemia cases (pre-T-ALL) are E rosette and OKT11 negative and only detectable by their reactivity with 3A1.¹⁶

The observation of nuclear blebs in both lymphoblasts and myeloblasts by electron microscopy in this case is another interesting feature. These structures have been previously identified in leukaemias and, infrequently, in normal blood cells. In general, they represent the ultrastructural expression of disturbances of DNA metabolism or nuclear membrane formation or both. In acute leukaemia a correlation between aneuploidy and a high frequency of nuclear blebs has been reported.¹⁷

Previously reported cases of mixed lymphoblastic myeloid leukaemia, either arising de novo or related to treatment, usually have lymphoblasts of the common or null cell phenotype.^{18–22} There are a number of case reports describing the sequential occurrence of a T cell leukaemia and acute or chronic myeloid leukaemia,^{23–25} although we believe

that this is the first reported case of a de novo mixed acute leukaemia in which there is evidence of T cell differentiation in the lymphoid lineage.

The association of a myeloblastic and T lymphoblastic phenotype in this case could be attributed to the coexistence of two abnormal clones arising from two separate cells²⁶ or, alternatively, they may have arisen from a single progenitor cell with differentiation along two lineages. The recognition of biphenotypic leukaemias is important so that appropriate chemotherapy can be given. In leukaemias with potential for differentiation along two pathways, cytotoxic treatment aimed specifically at one lineage may not be curative and relapse may occur with blasts characteristic of the untreated lineage.²⁷ Our patient was treated with a standard treatment for adult acute lymphoblastic leukaemia (UKALL IX regimen) that incorporates daunorubicin, vincristine, and prednisolone. It is of interest that a complete remission was achieved and maintained with treatment that is not totally appropriate for acute myeloid leukaemia. This study also confirms the value of immunological studies and electron microscopy in the characterisation of mixed leukaemias.

We thank Dr DJ Maberly, Airedale General Hospital, and Dr AG Bynoe for their help in this investigation. JTC was supported by the Calouste Gulbenkian Foundation, Portugal, and NP by the Associazione Italiana per la Ricerca sui Cancro.

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Requests for reprints to: Dr CS Scott, Department of Haematology, Regional Radiotherapy Centre, Cookridge Hospital, Leeds LS16 6QB, England.