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

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SUMMARY A case of mixed acute leukaemia with T lymphoblastic, myeloblastic, and monocytic components is described. The use of immunological markers, ultrastructural morphology, cytochemistry, immunocytochemistry, 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 × 10⁹/l (differential 98% blasts, 2% lymphocytes, with an occasional granulocyte) and platelet count 65 × 10⁹/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)
Precursor cells

Marker % Positive cells Specificity Source Reference

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 Ortho 13
UCHT1 5 Helper T cells Dr P Beverley 31
OKT4 <1 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
LexM3 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
M6A <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.

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 remaining having the typical acid phosphatase focal positivity suggestive of T cell acute lymphoblastic leukaemia blasts.
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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 × 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 × 14 700.
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 myeloperoxidase reactive granules and mitochondria. Some of the myeloid cells showed monocyteic 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 3Al, 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. 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...
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.

3AI positive cells invariably corresponded to blasts with lymphoid morphology. 3AI 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. 3AI 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 3AI 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 3AI reactive cells as non-myeloid (myeloperoxidase negative) is important because 3AI 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, 3AI, 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 3AI.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 cells 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. 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.

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References


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