Megakaryoblastic transformation of chronic granulocytic leukaemia

An electron microscopy and cytochemical study

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SUMMARY Morphological, cytochemical, and ultrastructural electron microscopic (EM) studies were performed on blood and bone-marrow cells of a case of Ph¹-positive chronic granulocytic leukaemia in megakaryocytic acute transformation. The entire leukaemic cell population was found to consist of megakaryoblasts and megakaryocytes. Intermediate stages of maturation between blasts and micromegakaryocytes were observed at EM level.

Micromegakaryocytes, usually mononuclear or binuclear, have been recognised as a significant component of the bone marrow in acute myeloid leukaemia (AML), in the preleukaemic stage of AML, and in refractory anaemia with an excess of myeloblasts. In the patient reported here virtual replacement of the marrow by micromegakaryocytes was the chief manifestation of the transformed stage of Philadelphia positive chronic granulocytic leukaemia (CGL). This case accents the fact that CGL is a stem-cell defect, rather than a defect only of granulocytic precursors, and is a further illustration of the failure of megakaryocyte polyploidisation in myeloid malignancy.

Case report

The patient (IS) was a 44-year-old schoolteacher who had been in good health until January 1975, when she presented for medical attention because of increasing abdominal girth. She gave a history of two months' lethargy, dyspnoea, and mild ankle swelling. She had suffered occasional night sweats. Her only relevant past history was of phlebitis in the right leg one year previously. On examination she was pale and the spleen was enlarged 18 cm below the left costal margin; the liver was just palpable.

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Haematological findings

The haemoglobin (Hb) was 6·6 g/dl and the leucocyte count was 290 × 10⁹/l with 1% blasts, 7% promyelocytes, 17% myelocytes, 25% metamyelocytes, 39% neutrophils, 3% basophils, and 3% eosinophils. There was one nucleated red cell per 100 white blood cells. The neutrophil alkaline phosphatase score (NAP) was 2. Direct examination of the peripheral blood revealed the presence of the Philadelphia chromosome (Ph¹). A bone marrow aspirate showed the marrow to be markedly hypercellular with hyperplasia of neutrophil, eosinophil, and basophil lines. Megakaryocytes were increased and an increased percentage of mononuclear and non-segmented forms was noted. A diagnosis of chronic granulocytic leukaemia was made.

The initial treatment was by leucapheresis and peripheral blood leucocytes were frozen and stored in liquid nitrogen (Lowenthal et al., 1976). Subsequently, control was achieved with demecolcine for two months followed by busulphan. Seven months after presentation she was submitted to elective splenectomy as part of a programme to assess the place of splenectomy in chronic granulocytic leukaemia. Before splenectomy the patient was clinically well, and the liver and spleen were impalpable. The Hb was 13·3 g/dl, the leucocyte count was 6·4 × 10⁹/l, and the platelet count was 344 × 10⁹/l. Immature granulocytic cells and circulating erythroblasts were not seen in the peripheral blood.

After splenectomy the patient remained well but
two months later the blast count had exceeded the myelocyte count. Attempts at control with cyclophosphamide and melphalan were unsuccessful and there was a progressive fall of the haemoglobin and neutrophil count. The slow rise of blast cells was associated with the appearance in the blood of micromegakaryocytes of similar size to a normal lymphocyte, and of apparently bare nuclei. By February 1976, six months after splenectomy, haematological values were as follows: Hb 9·2 g/dl (post-transfusion); platelet count 190 \( \times \) 10\(^9\)/l; nucleated-cell count 66·8 \( \times \) 10\(^9\)/l with 32\% blasts, 62\% micromegakaryocytes (Fig. 1), and bare nuclei, 1\% myelocytes and 1\% neutrophils. There were no eosinophils, basophils or monocytes. Giant platelets were present. A bone-marrow aspirate showed an intensely hypercellular marrow; 35\% of the cells were apparently undifferentiated blasts, some of which had a few small azurophilic granules which were myeloperoxidase negative. Sixty per cent of the cells were megakaryoblasts and micromegakaryocytes. Some of these were producing platelets and some were almost bare of cytoplasm. Special studies were performed on bone-marrow and buffy-coat preparations at this time. Subsequently, attempts were made to ablate the acutely transformed marrow and to autograft stored, cryopreserved chronic-phase peripheral blood granulocytes. These attempts were unsuccessful and the patient finally died in March 1976, 15 months after presentation.

Methods

Standard methods were used for haematology, cytology, and ultrastructural studies. For ultrastructural cytology the myeloperoxidase method of Graham and Karnovsky (1966) was used; the modification to detect megakaryocyte peroxidase was not used (Breton Gorius et al., 1972). Cytochemical and ultrastructural studies were done on both bone-marrow aspirates and buffy-coat preparations. Immunological tests for B and T lymphocytes were performed. Cells were investigated by Dr M. Greaves (University College, London) using antisera raised against various types of acute leukaemia cells (Janossy et al., 1976).

Results

ELECTRON MICROSCOPY AND ULTRASTRUCTURAL CYTOCHEMISTRY (Figs. 2-7)
The majority of cells in both bone-marrow and buffy-coat preparations were identified as micromegakaryo-
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Cytes (Fig. 2). Nuclei were round or oval with moderately condensed chromatin, and in the most mature cells there was no nucleolus. The cytoplasm contained prominent demarcation membranes and dense osmiophillic granules including bull’s eye granules characteristic of the megakaryocytic series (Figs. 3 and 7). Mitochondria, free ribosomes, endoplasmic reticulin, and glycogen granules were present. Glycogen granules tended to be concentrated in the periphery of the cell. Some cells had numerous surface blebs, and glycogen granules were sometimes concentrated in these blebs. The cytoplasm of the micromegakaryocytes was similar in appearance to the giant platelets. Megakaryocytes commonly showed the appearance of myelin bodies and these were occasionally seen in platelets (Fig. 6).

The majority (about one-third) of the bone-marrow and peripheral-blood cells were blast cells (Fig. 4). The nucleus was slightly irregular with finely dispersed chromatin and usually a large nucleolus. The cytoplasm contained numerous mitochondria, abundant ribosomes, and long strands of endoplasmic reticulum. There were occasional granules and these were always peroxidase negative.

All stages of maturation were present between the blasts and the mature micromegakaryocytes (Figs. 3 and 5). There appeared to be a continuous spectrum of cells. In comparison with the blast cells, intermediate stages had a greater degree of chromatin condensation and less frequently had a nucleolus. With maturation there was a progressive decrease in the number of mitochondria with an increase in the number of dense granules and demarcation membranes. The continuous spectrum of cells, the myeloperoxidase negativity of the granules, and the presence of some blasts showing the early stages of formation of demarcation membranes suggested
Fig. 3  Electron microscopy of buffy coat showing the range of megakaryocyte differentiation from a megakaryoblast (mb) with occasional granules to a slightly more mature cell with more granules and demarcation membrane (centre) to micromegakaryocytes (mm) and giant platelets (p), some showing glycogen granules and bull's eyes granules. × 6000.

Fig. 4  Electron microscopy of bone marrow showing a megakaryoblast with diffuse chromatin pattern, mitochondria, a few dense granules, and long profiles of endoplasmic reticulum. × 11 000.
Fig. 5  Electron microscopy of bone marrow showing a cell intermediate between a megakaryoblast and a micromegakaryocyte. There is more condensation of chromatin, some demarcation membranes, and moderately numerous granules. × 10 000.

Fig. 6  Electron microscopy of bone marrow showing a micromegakaryocyte with myelin bodies. × 10 000.
that the relatively undifferentiated blasts were megakaryoblasts rather than myeloblasts or monoblasts. The apparently 'bare' nuclei seen on light microscopy were found on electron microscopy to have a thin rim of cytoplasm containing only a few granules and small amounts of the demarcation membrane system (Fig. 2).

CYTOCHEMISTRY
Myeloperoxidase, Sudan Black B, and the cytochemical tests for lysozyme gave negative results in both blasts and micromegakaryocytes. The PAS reaction showed heavy positivity with a finely granular pattern, particularly concentrated around the periphery of the cell which was the area most frequently showing glycogen granules on electron microscopy. Acid phosphatase was strongly positive in the blasts, the micromegakaryocytes, and the platelets. Naphthol AS Acetate Esterase (NASA) was strongly positive in blasts, micromegakaryocytes, and platelets. The reaction was moderately weakened by sodium fluoride. The pattern of positivity with the PAS, NASA, and acid phosphatase reactions was almost identical with the one observed in normal megakaryocytes.

IMMUNOLOGICAL STUDIES
The sheep red blood cell rosette test gave a negative result, and no surface immunoglobulins were detected. The cells did not react with an anti acute lymphoblastic leukaemia (ALL) serum or with an antigranulocyte serum; a weakly positive reaction was obtained with an anti-AML serum.

Discussion
Morphological, cytochemical, and ultrastructural studies of the blood and bone-marrow of this patient have shown that her bone-marrow was replaced by megakaryoblasts and micromegakaryocytes. The

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Fig. 7  Electron microscopy of bone marrow showing a micromegakaryocyte with abundant granules including bull's eye granules, demarcation membranes, and surface blebs. × 10 000.
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Micromegakaryocytes accounted for two-thirds of her bone-marrow cells, and they circulated freely in her peripheral blood. On light microscopy many of them appeared bare or almost bare of cytoplasm, but on electron microscopy all had a thin rim of cytoplasm. Both megakaryocytes and more primitive cells were producing platelets, many of which were giant forms with diameters of up to 6 µm. There were no recognisable myeloblasts in the marrow. Some blasts had fine azurophilic granules but none showed positivity to Sudan Black B staining, and the myeloperoxidase reaction was negative even on electron microscopy.

In the chronic stage of CGL the average size of megakaryocytes is less than normal and this may be associated with decreased megakaryocyte ploidy (Albrecht and Fülle, 1974). However, the proportion of micromegakaryocytes is small. Significant numbers of micromegakaryocytes have been described in preleukaemia (Queisser et al., 1972; Smith et al., 1973; Saarni and Linman, 1973), AML (Albrecht and Fülle, 1974), and refractory anaemia with an excess of myeloblasts (Breton Gorius et al., 1972). Breton Gorius has also described this phenomenon in a case designated as acute myelofibrosis (Breton Gorius et al., 1973), and Popescu (1974) has described it in four patients with sideroblastic anaemia, two of whom subsequently developed AML. In all these conditions in which the granulocytic and erythroid series are abnormal, the associated defective polyploidisation of the megakaryocyte line suggests an underlying stem-cell defect. In the chronic phase of myelofibrosis small micromegakaryocytes are commonly found in the peripheral blood, and after splenectomy they may reach a significant percentage (Carpenter and Flory, 1941). However, the size and nuclear form of tissue megakaryocytes are relatively normal.

In the transformed stage of CGL a small proportion of the blasts may be micromegakaryoblasts (Castoldi et al., 1975). In this present case the megakaryoblasts and micromegakaryocytes made up the entire population of the marrow during the stage of blastic transformation of CGL. The appearance of myelin bodies in platelets in association with micromegakaryocytes has been noted also by Breton Gorius et al. (1973).

CGL arises by mutation in a pluripotential myeloid stem cell. Although the major clinical manifestations reflect the proliferation of the granulocyte series, both G-6-PD and 6-phosphogluconate dehydrogenase isoenzyme studies and the presence of the marker Ph1 chromosome support the origin of the abnormal clone from a common precursor of the granulocytic, megakaryocytic, and erythroid cell lines (Fialkow, 1974; Fialkow, 1975). CGL usually terminates by transformation to a blastic stage. The blasts are usually myeloblasts, and at least some have azurophilic granules which are myeloperoxidase and Sudan Black B positive, although Auer rods are rare. In a significant minority the blasts are agranular forms; they react with antisera raised against childhood ALL cells, and may contain the thymic enzyme terminal deoxynucleotidyl transferase (Janossy et al., 1976; McCaffrey et al., 1975). A therapeutic response to prednisolone is more likely in patients with agranular blasts than in those with myeloblasts (Marmont and Damasio, 1973). Boggs (1974) discusses the implications of these lymphoblastic-like transformations in relation to the nature of the stem cell involved in CGL. Erythroblastic and monoblastic transformations are uncommon. Srodes et al. (1973) described two patients with typical Ph1 positive CGL who underwent erythroblastic transformation with megakaryoblastic erythropoiesis. Megakaryoblastic transformation is similarly uncommon. Minot (1922) described the appearance of up to 18% of small megakaryocytes in the peripheral blood of a patient with CGL in the chronic phase, and also noted that the appearance of megakaryocytes in the peripheral blood may be the first sign of increased activity of the disease; two patients who had up to 8% of megakaryocytes in the peripheral blood died within three months. Ogawa et al. (1970) refer to the terminal phase of typical CGL in which 90% of circulating 'white cells' were abnormal megakaryocytes and fragments thereof. Maldonado (1974) described a case of Ph1 positive 'agnogenic myeloid metaplasia': there were dysplastic platelets in the peripheral blood and 20% of the cells were micromegakaryocytes; there were numerous megakaryocytes in the marrow. Marrow fibrosis is a well-known occurrence in CGL, and this case may have been one of atypical CGL in megakaryocytic transformation. In another of his cases of typical Ph1 positive CGL the same morphological findings were observed, but only 1-2% of the circulating cells were micromegakaryocytes. In the case of Ph1-positive megakaryoblastic leukaemia reported by Hossfeld et al. (1975), basophilia and bone-marrow myeloblastosis were noted during the course of the disease, and this case may have been CGL presenting in transformation. There was thrombocytosis, and between 20 and 46% of bone marrow cells were megakaryocytes, mostly diploid. Cases of primary haemorrhagic thrombocytopenia or megakaryocytic myelosis with a Ph1 chromosome (Tough et al., 1963; Dougan et al., 1967; Ghosh, 1972) represent a more chronic related disorder which is probably an uncommon variant of CGL in which megakaryocytic proliferation is unusually prominent. The transformation to acute leukaemia which occurred in one case (Dougan et al., 1967) again emphasises the stem-cell
defect present in this condition.

Cytological study of the blast cells of transformed CGL may be of practical therapeutic value and is also of great theoretical interest. Thus the occurrence of megakaryoblastic and erythroblast transformation is further evidence supporting that derived from cytogenetics and biochemistry, that the disease involves a pluripotential stem cell. The recent evidence of Janossy et al. (1976) suggests that at least in some cases the mutation may occur in a stem cell which has the capacity for both myeloid and lymphoid differentiation.

References


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