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Maturation of Blast Cells in Acute Transformation of Chronic Myeloproliferative Syndrome* **

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In recent years, data have accumulated which demonstrate that the failure of human leukaemic blast cells to differentiate is not an inherent defect, but rather one that can be overcome under in vitro culture conditions. This has been established both for cell lines and for fresh human leukaemic blast cells (LBC). The differentiation of human LBC into different cell pathways is possible. Differentiation to more mature stages has been demonstrated, into granulopoietic cells for the K60 human promyelocytic line (Collins et al. 1977) and for the K61 cell line (Koeffler et al. 1978), into cells with erythropoietic features for the K562 cell line (Anderssen et al. 1979), and even into lymphopoiesis, as for the Reh cell line (Lau et al. 1979).

The maturation of fresh human LBC from some cases of acute myeloid leukaemia (AML) into granulopoietic cells or macrophages has also been observed during in vitro culture as a liquid suspension or in a agar system. When AML blast cells are cultured in diffusion chambers (DC), a terminal differentiation into mature granulocytes is observed in half of the patients, and these cells show partially normal function when tested for phagocytosis or by nitrotetrazolium blue reduction (Hoelzer et al. 1980).

In the present study, blast cells from patients in the blast crisis of chronic myelocytic leukaemia (CML) were investigated for their ability to differentiate in diffusion chamber culture. The aim was to see whether terminal differentiation into mature granulocytes occurs, as in AML, and whether differentiation into other haemotological cell lines is also possible, which might indicate that blast cells in CML blast crisis are primitive cells and therefore able to differentiate into haemopoietic pathways other than granulopoiesis.

A. Materials and Methods

The material was provided in a cooperative study on the pathophysiology of CML blast crisis by the Süddeutsche Hämoblastosegruppe. The blast cells in CML blast crisis were characterized by cytochemistry (PAS, POX, esterase) and by immunological cell surface markers. The findings presented in this paper relate to cells from 24 patients showing the myeloid type of blast crisis.

Peripheral blood cells were separated by centrifugation on an Isopaque-Ficoll gradient resulting in an enrichment of blast cells to over 80% in most cases. The diffusion chamber technique was carried out as described previously (Hoelzer et al. 1977). In brief, DC were filled with 5×10^5 nucleated cells, implanted into host mice pre-irradiated with 750 rad and cultured for up to 8 weeks. The chambers were removed every week, cleaned with sterile gauze to remove the host cells coating the membranes and re-implanted into fresh, irradiated hosts. Chamber contents were harvested at weekly intervals and investigated for total and differential cell counts and for cytochemical tests, including peroxidase, PAS, naphthol-AS-acetate esterase and leucocyte alkaline phosphatase, and for CFU-C content cytogenetics, phagocytosis and NTB reduction.

B. Results

When leukaemic blood cells from patients with blast crisis in CML were cultured in diffusion

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chambers, the growth pattern observed was quite distinct from that for cells from the chronic phase of CML and also from that for normal peripheral blood cells. For most of the patients in blast crisis, there was a net increase in total cell number which was almost continuous, reaching values greatly in excess of normal. This rise in cellularity was due to proliferation of blast cells and a considerable degree of maturation of DC cells into proliferating and non-proliferating granulopoiesis As shown in Fig. 1, the increase in proliferating granulopoietic cells, i.e. promyelocytes and myelocytes, was immediate. Mature granulopoietic cells decreased initially in most cases, most probably owing to death. A rise in mature granulopoietic cells was delayed, usually for about 1 week, until cells newly developed from the immature stages appeared (Fig. 2). In 22 of 24 cases the values reached for granulopoietic cells were well above the levels for normal peripheral blood cells cultured in DC, as well as above the levels for CML in the chronic phase.

Development of megakaryocytes was observed in 16 of the 24 patients studied who had CML blast crisis. New erythropoietic cells were seen in 13 of the 24 cases. Lymphocytes were fewer and plasma cells scarce in comparison with those found in cultures of mononuclear blood cells from normal persons.

C. Discussion

The experiments described here show that when blast cells from patients with blast crisis in CML are cultured in diffusion chambers for periods of up to 8 weeks, there is a marked proliferation of blast cells and a new development of granulopoietic cells together with some megakaryocytes and erythropoietic cells. The differentiation into granulopoietic cells is not restricted to the immature forms, but terminal differentiation into mature granulocytes could also be observed in 80% of the patients studied. The origin of these differentiated cells is a crucial point. They might be derived from the implanted blast cells, from Ph' positive stem cells typical of the chronic phase of CML or even from remaining normal stem cells. Arguments in favour of an at least

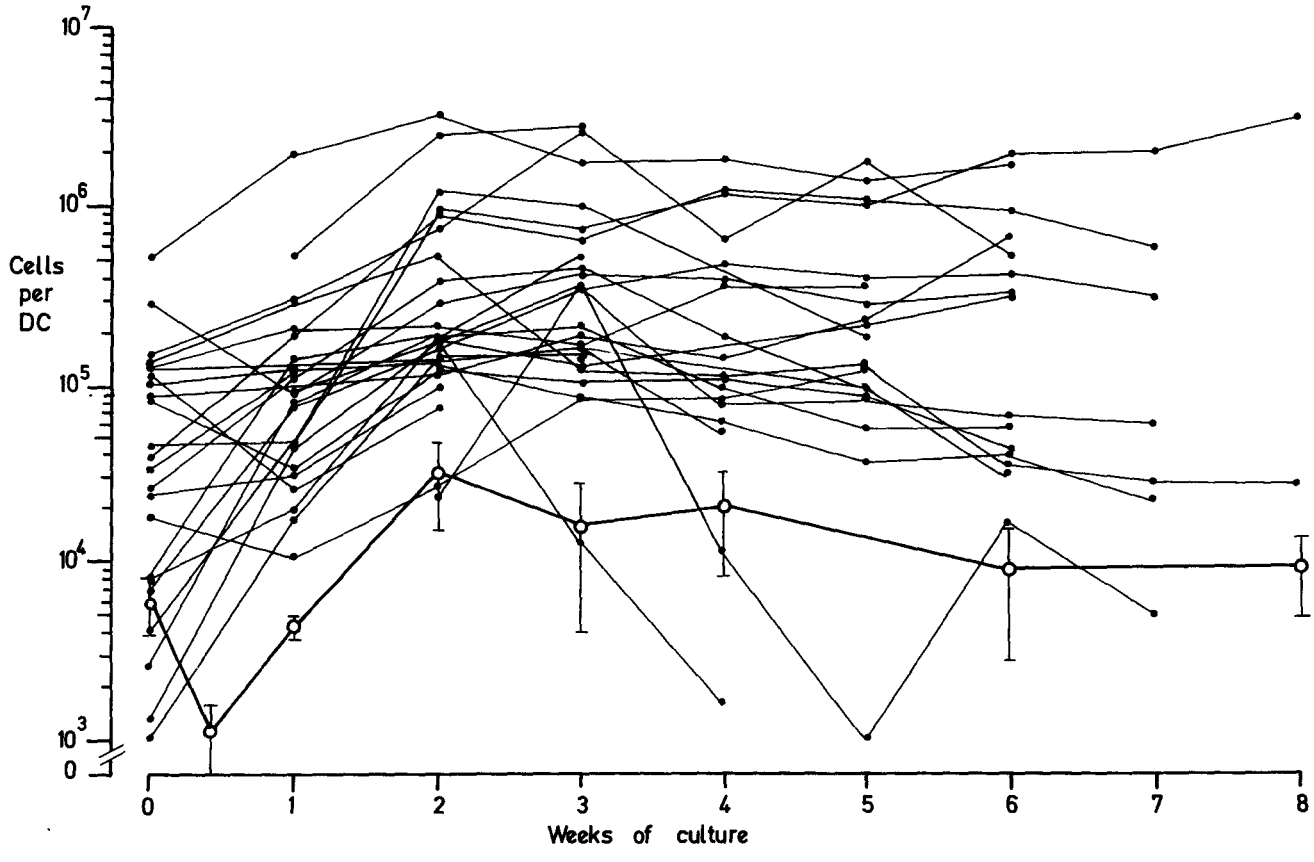

Fig. 1. Change in number of proliferating granulopoietic cells harvested from diffusion chambers at weekly intervals. Results from 24 patients with blast crisis in chronic myeloid leukaemia. *Open Circles* and *bars* denote mean values ± S.E.M. for *DC* growth of mononuclear blood cells from normal persons

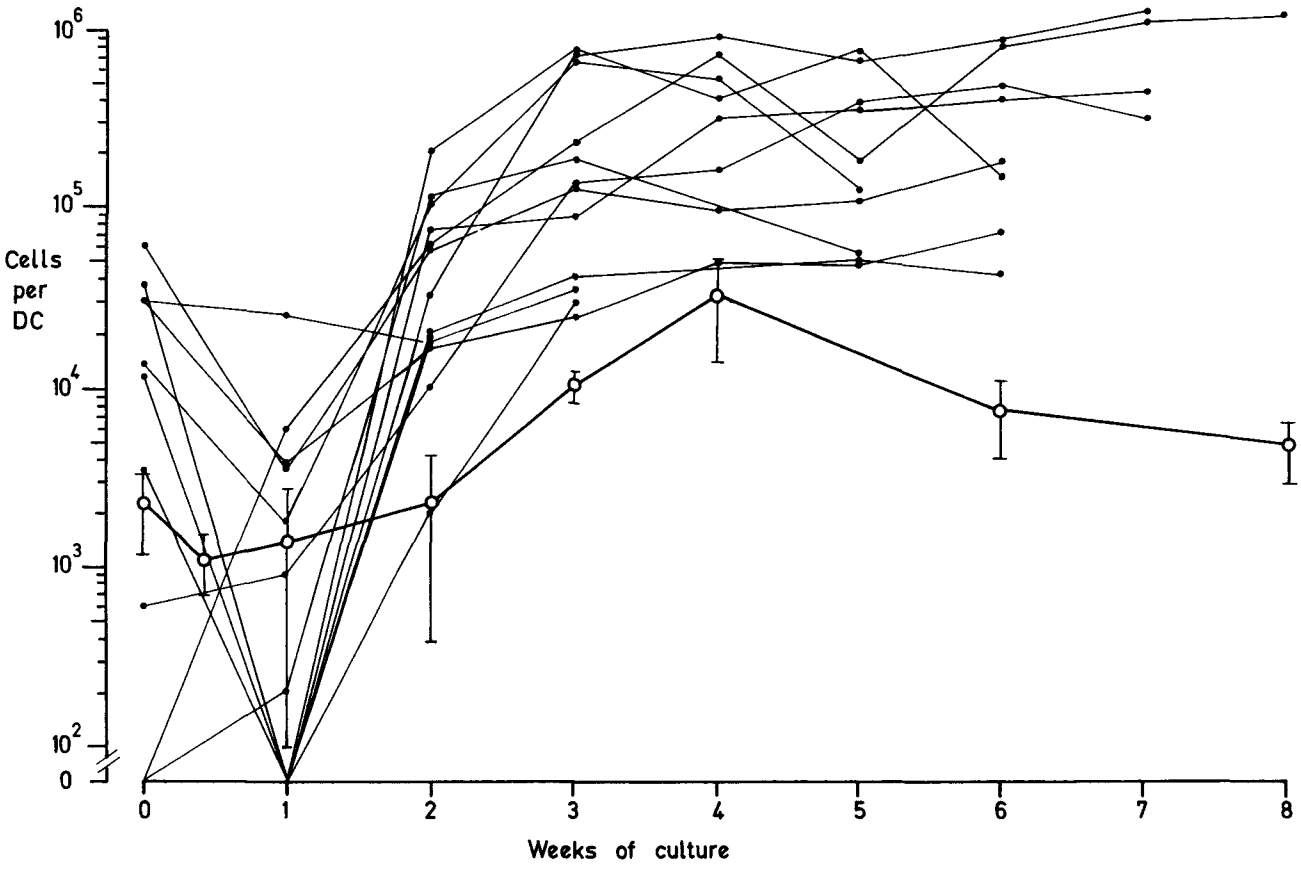

Fig. 2. Change in number of non-proliferating granulopoietic cells. Details as in Fig. 1

partial origin from blast cells are the fact that the cell suspension used for culture consisted of more than 90% blast cells in some cases, the growth pattern of the cells in culture and the results of cytogenetic analysis.

The growth pattern for CML blast crisis cells in DC culture with continuous proliferation and differentiation resulting in a progressive increase in total cell number, in some cases for the whole 8-week culture period, differs strikingly from the growth pattern for CML cells from the chronic phase. In the latter, although proliferation and differentiation into granulopoietic cells were also observed, the constant decline in total cellularity, as found also by Chikkappa et al. (1973), indicated that Ph' positive stem cells in the chronic phase apparently do not possess the potential for unlimited proliferation under these culture conditions.

In the patients studied who had CML blast crisis, the cells harvested from DC and subjected to cytogenetic analysis were Ph' positive and some cases also showed additional chromosome aberrations characteristic of acute transformation in CML. There were, however, five cases of CML blast crisis in which *all* the DC cells tested had, as well as the Ph' chromosome, an additional clonal aberration, indicating that the proliferating cells in these DC cultures were derived from that abnormal clone. It might, therefore be concluded that the mature cells are also descendants of these clones.

A noteworthy finding is the proportion of newly developed megakaryocytes and erythropoiesis from CML blast crisis cells, which was much higher than that developing from chronic phase cells in DC. Although it seems likely, there is as yet no certain proof that either the megakaryocytes or erythropoietic cells are descendants of the blast cells in CML blast crisis and are not descendants from remaining chronic phase cells. From the former possibility one could speculate that blast cells in the myeloid type of blast crisis possess a greater potential for differentiation into different cell lines than do AML cells. This in turn would mean the transformation of an early precursor cell, similar to that known from studies of CML in the chronic phase, where involvement of granulopoiesis, erythropoiesis and megakaryocytopoiesis has been demonstrated (Fialkow, 1979).

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