Differentiation Ability of Peripheral Blood Cells from Patients with Acute Leukemia or Blast Crisis in Chronic Myelocytic Leukemia*

Hoelzer, D.,[1], Harriss, E. B.[1], Kurrle, E.[1], Schmücker, H.[1], Hellriegel, K. P.[2]

1 Depts. of Internal Medicine and Clinical Physiology, University of Ulm, 7900 Ulm, Federal Republic of Germany
2 Dept. of Internal Medicine, University of Köln, 5000 Köln, Federal Republic of Germany

Introduction

The diffusion chamber (DC) system of in vivo culture is useful for testing human leukemic peripheral blood cells for several reasons. With this technique, stem cells apparently preceding the granulopoietic progenitor cells (CFU-c) can be detected. Arguments for this come from diffusion chamber studies on normal human peripheral blood cells where an absolute increase in CFU-c was found (Hoelzer et al., 1976a) and also from DC culture of human bone marrow where CFU-d (cells that form granulocytic colonies in DC) as precursors of CFU-c could be distinguished on the basis of different velocity sedimentation profiles and different ^{3}H-thymidine suicidal fractions (Jacobsen et al., 1978). Differentiation of cells in the DC system occurs not only into the granulopoietic/macrophage series but also into megakaryocytes and, even if rarely, into erythropoiesis, as well as into lymphatic cell lines. Furthermore, the cultures can be maintained over long periods; in our present experiments leukemic cells have been cultured for up to 2 months and Hodgkin cells for even longer (Boecker et al., 1975). Thus the DC system seems to be suitable for sustaining the growth of leukemic cells of an earlier "stemness" than normal CFU-c and seems also to promote differentiation into haemopoietic cell lines other than granulopoiesis.

On this basis, the DC culture is here used to study the differentiation ability of peripheral blood cells from patients with various forms of acute leukemia or with blast crisis in chronic myelocytic leukemia (CML).

Materials and Methods

Peripheral blood samples obtained from patients with acute myelocytic leukemia (AML), acute lymphoblastic leukemia (ALL), acute undifferentiated leukemia (AUL) or blast crisis in CML were separated by the Isopaque-Ficoll technique and the mononuclear cell fraction was cultured in DC as already described in detail (Hoelzer et al., 1974). In most cases, blast cells constituted more than 80% of this fraction, the rest being mainly lymphocytes.

---

* Work was supported by the Sonderforschungsbereich 112, Project B3

The cells (5×10^5 in 0.1 ml) were filled into DC with filter pore size 0.22 μm implanted into the peritoneal cavity of mice pre-irradiated with 650 R or 750 R and the chambers were then re-implanted weekly into new pre-irradiated recipients. In harvesting, the clot formed in DC was dissolved by pronase treatment and from the resulting single cell suspension the total nucleated cell number per chamber was determined, smears were made and stained with Giemsa or peroxidase reaction, an aliquot prepared for cytogenetic analysis and a further aliquot used for assay of CFU-c content by agar colony culture.

Results

The total growth of nucleated cells in DC of AML blood cells was in general well above normal values, whereas growth from ALL cells is much less than normal (Hoelzer et al., 1977). In contrast to the normal pattern, which has an initial decrease in total cell number and an increase between days 6 and 13 of culture, for most cases of AML the cell number starts to increase immediately after implantation and continues to increase over the whole culture period, in some cases up to 6 or 7 weeks. There is not only a proliferation of blast cells, as demonstrated by ^{3}H-thymidine studies (Hoelzer et al., 1976b), but also a development of large numbers of granulopoietic cells.

The absolute number of granulopoietic cells developing from AML cells was far in excess of that from normal blood cells (Fig. 1), except in one pa-

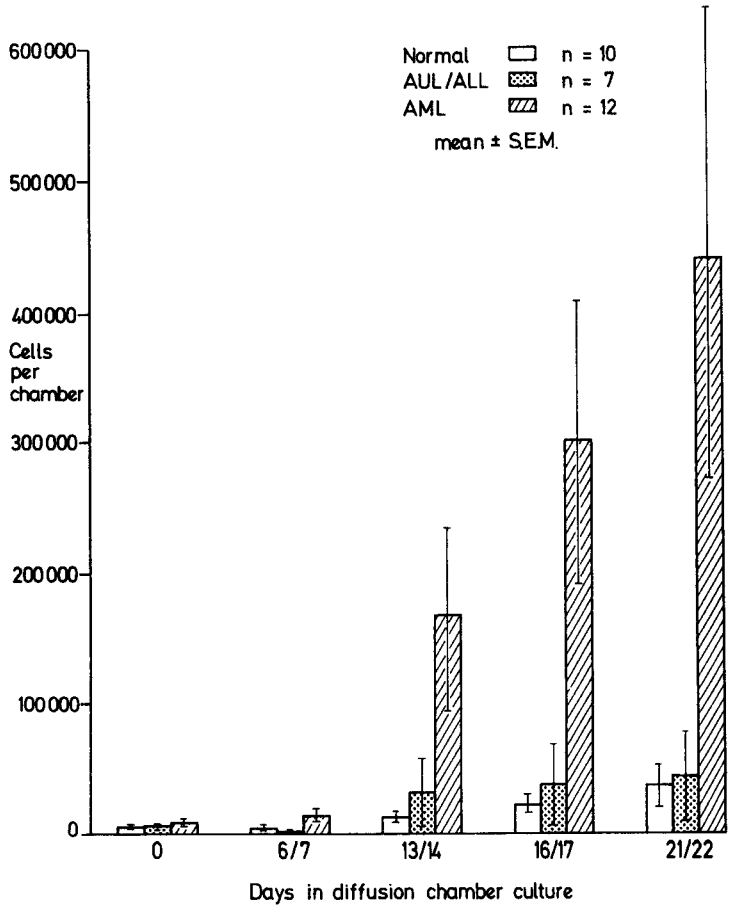

Fig. 1. Absolute numbers of granulopoietic cells per diffusion chamber at the time of implantation (day 0) and during culture for peripheral blood cells from normal persons and for patients with AML or AUL/ALL

tient. Sometimes the newly appearing granulopoietic cells were of abnormal morphology, but their granulopoietic nature could be confirmed by the peroxidase reaction or Sudan black staining. Although in some cases maturation was restricted to the myelocyte stage, in 6 out of 12 cases of AML terminal differentiation up to segmented granulocytes was seen, exceeding the numbers from normal persons. In addition, cells from one AML patient developed predominantly into mature eosinophils.

In cultures of blood cells from patients with ALL or AUL, the number of granulopoietic cells which developed was of the same order as from normal blood cells, and it might well be that they were descendants of normal stem cells which had been suppressed in vivo but could grow in DC culture conditions.

Peripheral blood cells from patients with *blast crisis in CML* had a similar growth pattern to AML cells. As shown in Fig. 2, there was a continuous pro-

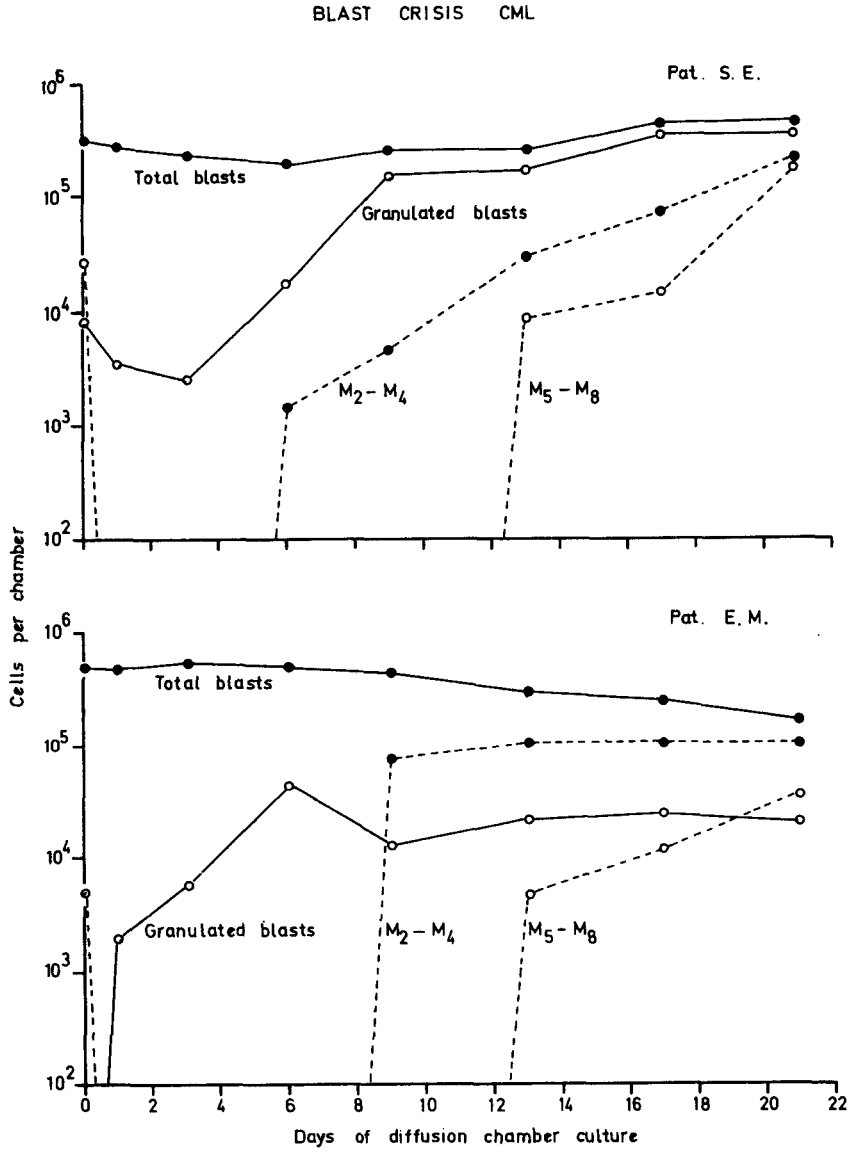

Fig. 2. Growth in diffusion chambers of peripheral blood cells from two patients with blast crisis in CML. The numbers of blast cells, appearance of granulated blast cells (immature blast cells with azurophilic granules in the cytoplasm), immature proliferating (M_2–M_4) and mature (M_5–M_8) granulopoietic cells are given

liferation of blast cells. Granulated blast cells appeared in one patient (E. M.) and increased soon after implantation in the second patient (S. E.). The immature granulopoietic precursors (M_2–M_4) in the cell inoculum disappeared immediately after implantation and reappeared within a week; after about 12 days of culture, mature granulopoietic cells (M_5–M_8) were observed. From the sequence of events it seems likely that the blast cells renewed themselves and also differentiated into granulated blasts with subsequent differentiation into immature and finally mature granulopoietic cells.

Discussion

The principal point of these investigations is whether the granulopoietic cells which developed from AML cells were derived from leukemic cells or from remaining normal stem cells. Although there is no conclusive proof that they were leukemic descendants, several arguments strongly indicate that they were at least partially of leukemic origin.

The main argument is the *absolute number of granulopoietic cells* from patients with AML, exceeding normal values by up to 40-fold. In most of these cases, the cell inoculum comprised over 80% blast cells, so that an exclusively normal origin for the granulopoietic cells would require a vastly increased proportion of normal circulating stem cells in the lymphocyte population, a somewhat unlikely event for leukemic patients. A further consideration is the *early appearance* of granulopoietic cells in cultures of AML cells. Whereas from normal blood cells, granulopoiesis was not observed before day 5, in some cultures of AML cells granulopoietic cells appeared almost immediately (days 1–3) after implantation. It must therefore be assumed that leukemic cells, although of immature morphology, require only one or two steps of division to become recognisable granulopoietic cells.

The *cytogenetic analyses* contribute only partial evidence for the leukemic origin of the granulopoietic cells. The cells harvested from DC showed hyperdiploid marker chromosomes in 5 out of 13 patients. However, it should be mentioned that in all cases numerous diploid metaphases were present and also hypodiploid metaphases, the latter being probably technical artefacts.

With other culture systems it has been demonstrated in recent years that differentiation is possible in some cases of AML. Reports of this in the agar colony culture system with an exogenous source of stimulation come from Paran et al. (1970), Robinson and Pike (1971), and Barak et al. (1974) although often with abnormal morphology (Moore et al., 1973). A certain extent of differentiation was also observed in the liquid culture system (Gold and Cline, 1973). In continuous long-term culture, one AML cell line (H-60) was established which showed continuous differentiation into granulopoiesis without additional stimulation (Collins et al., 1977) and the authors suggest that a sub-population of leukemic blast cells might secrete factors regulating proliferation and differentiation. In the diffusion chamber culture system, other workers have failed to demonstrate differentiation from leukemic bone

marrow cells (Fauerholdt and Jacobsen, 1975; Steele et al., 1977) and it is open to question whether this might have been due to inhibition by a particular fraction of bone marrow cells, to inadequate stimulation of the host animals by a lower radiation dose or to the use of cyclophosphamide. However, in DC culture of peripheral blood cells from a patient with AML maturation of leukemic cells could be confirmed by the presence of Auer rods in the non-proliferating granulopoietic cells (Boecker et al., 1978).

The investigation described here provides evidence that in nearly all cases of AML, differentiation into recognisable granulopoiesis is possible, with terminal differentiation up to mature neutrophils in half the patients studied. Similarly, it seems that in the myeloid type of blast crisis in CML a differentiation into the granulocytic series can occur.

References

Barak, Y., Shore, N.A., Higgins, G.R., Vadakan, V.: Functional cellular maturation in cultures of human haematopoietic cells. Brit. J. Haematol. **27,** 543–549 (1974)

Boecker, W.R., Hossfeld, D.K., Gallmeier, W.M., Schmidt, C.G.: Clonal growth of Hodgkin cells. Nature **258,** 235–236 (1975)

Boecker, W.R., Oehl, S., Hossfeld, D.K., Schmidt, C.G.: Differentiation of Auer-rod positive leukemic cells in diffusion chamber culture. Lancet **1978 I,** 267–269

Collins, S.J., Gallo, R.C., Gallagher, R.E.: Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. Nature **270,** 347–349 (1977)

Fauerholdt, L., Jacobson, N.: Cultivation of leukemic bone marrow cells in diffusion chambers implanted into normal and irradiated mice. Blood **45,** 495–501 (1975)

Golde, D.W., Cline, M.J.: Growth of human bone marrow in liquid culture. Blood **41,** 45–57 (1973)

Hoelzer, D., Kurrle, E., Ertl, U., Milewski, A.: Growth of human leukemic peripheral blood cells in diffusion chambers. Eur. J. Cancer **10,** 579–589 (1974)

Hoelzer, D., Harriss, E.B., Slade, M., Kurrle, E.: Growth of in vitro colony forming cells from human peripheral blood leukocytes cultured in diffusion chambers. J. Cell. Physiol. **89,** 89–100 (1976a)

Hoelzer, D., Schmücker, H., Kurrle, E.: Re-entry of resting leukemic blood cells into proliferation in human acute leukemia during diffusion chamber culture. Blut **33,** 301–312 (1976b)

Hoelzer, D., Kurrle, E., Schmücker, H., Harriss, E.B.: Evidence for differentiation of human leukemic blood cells in diffusion chamber culture. Blood **49,** 729–744 (1977)

Jacobson, N., Broxmeyer, H.E., Grossbard, E., Moore, A.S.: Diversity of human granulopoietic precursor cells: Separation of cells that form colonies in diffusion chambers (CFU-d) from populations of colony-forming cells in vitro (CFU-c) by velocity sedimentation. Blood **52,** 221–232 (1978)

Moore, M.A.S., Metcalf, D.: Cytogenetic analysis of human acute and chronic myeloid leukemic cells cloned in agar culture. Int. J. Cancer **11,** 143–152 (1973)

Paran, M., Sachs, L., Barak, Y., Resnitzky, P.: In vitro induction of granulocyte differentiation in hematopoietic cells from leukemic and non-leukemic patients. Proc. Natl. Acad. Sci. USA **67,** 1542–1549 (1970)

Robinson, W.A., Kurnick, J.E., Pike, B.L.: Colony growth of human leukemic peripheral blood cells in vitro. Blood **38,** 500–508 (1971)

Steele, A.A., Sensenbrenner, L.L., Young, M.G.: Growth and differentiation of normal and leukemic human bone marrow cells cultured in diffusion chambers. Exp. Hematol. **5,** 199–210 (1977)