Proliferative Behavior of Hemopoietic Cells in Preleukemia and Overt Leukemia Observed in One Patient*

P. Dörmer

Institute of Hematology of the Gesellschaft für Strahlen- und Umweltforschung, München

Summary

Hemopoietic cell proliferation was studied in a patient suffering from preleukemia characterized by peripheral pancytopenia and hypercellular bone marrow with ineffective erythropoiesis. Two years later when overt acute myelogenous leukemia had developed the study was repeated. The kinetics of proliferation were investigated by a new method which allows evaluation of the rate and time of DNA synthesis in individual morphologically defined cells.

Erythropoiesis was found ineffective to the same degree in both stages of disease. The rate of erythroid cell proliferation, however, was reduced in overt leukemia only. The myeloid system showed a grossly reduced production rate of myeloblasts in preleukemia whilst the same parameter was strongly increased in leukemia. This high production rate of myeloblasts in overt leukemia was interpreted as indication of a far-reaching self-maintenance of the myeloblast pool in this stage of disease. The proliferative activity of the individual myeloblasts was reduced already in preleukemia, and even more so in leukemia. In order to explain the amplification of the myeloblast pool with the onset of overt leukemia a change in the mode of myeloblast divisions is assumed. For this a transition from steady state to some degree of exponential growth gives the most plausible explanation.

A 71 year-old female suffered from severe peripheral pancytopenia with an anemia of 7.9 gm % of hemoglobin, leukopenia of 1,240/mm^3 and thrombocytopenia of 24,000/mm^3. The anemia was classified as refractory anemia. The bone marrow was hypercellular with a G:E ratio of 1:2. 69 % of the erythroblasts were ringed sideroblasts according to the Prussian blue reaction. By ferrokinetic examination a highly ineffective erythropoiesis was found with a P.I.T of 3.55 mg Fe/100ml/day whilst the red blood cell lifespan turned out to be normal.

After unsuccessful therapy with vitamins B_6, B_{12} and folic acid the patient received merely occasional transfusions of packed red blood cells. She remained under out-patient control and did not show significant changes for the next 20 months. Then a gradual rise in the white blood cell count with an increasing number of myeloblasts in the blood smear was observed. 2 years after the first examination overt acute myelogenous leukemia (AML) had developed. Retrospectively the phase of pancytopenia was classified as preleukemia.

* Study performed under the association contract for hematology between EURATOM and GSF no. 089 72I BIAD. Supported by the Deutsche Forschungsgemeinschaft: SFB 51/E-3.

In the preleukemic state and at the stage of untreated AML sternal bone marrow was aspirated for the study of cellular proliferation. The suspended cells were incubated in a short-term incubation schedule with ^{14}C-thymidine (^{14}C-TdR) and 5-fluorodeoxyuridine (FUdR). By means of quantitative ^{14}C-autoradiography the duration of DNA synthesis (t_s) was evaluated in individual cells. The method as well as the pertinent principles of cell proliferation kinetics have been discussed in detail elsewhere (2). By Feulgen microphotometry euploid DNA values were obtained for the leukemic myeloblasts.

Preleukemia

	Pr. E.	Bas.E.	Pol. E.
N_C (rel.)	163	334	503
N_S/N_C	0.78	0.70	0.32
t_S (h)	9.1	13.1	16.2
N_S/t_S (rel.)	1.0	1.3	0.7

AML

	Pr. E.	Bas.E.	Pol.E.
N_C (rel.)	110	283	607
N_S/N_C	0.69	0.56	0.24
t_S (h)	10.3	15.5	23.0
N_S/t_S (rel.)	1.0	1.4	0.9

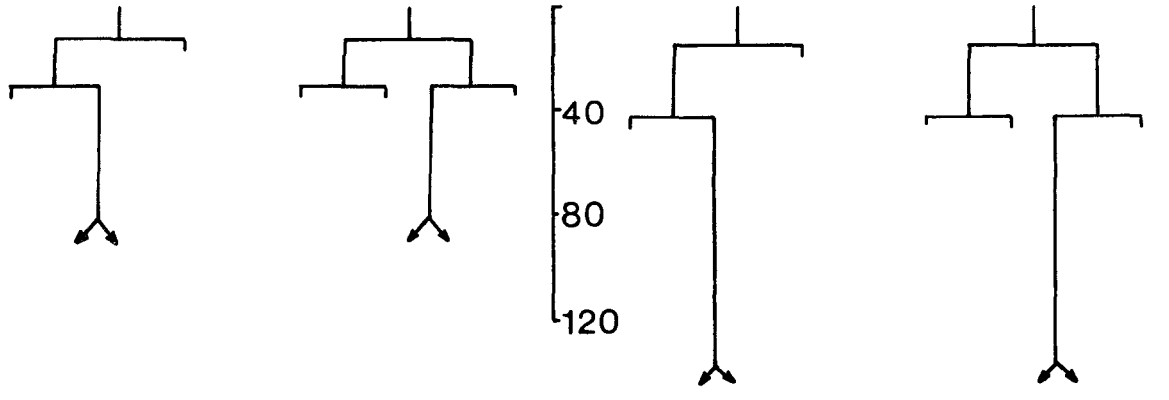

Fig. 1: Parameters of cell kinetics and schemes of divisions of erythroid cells in preleukemia (left side) and AML (right side). Between the schemes of divisions a time scale in hours is inserted. Abbreviations: Pr. E. = proerythroblasts; Bas. E. = basophilic erythroblasts; Pol. E. = polychromatic erythroblasts; N_c = relative number of cells in a compartment; N_s/N_c = ^{3}H-TdR labeling index; t_s = DNA synthesis time; N_s/t_s = relative rate of cell production in a compartment.

Fig. 1 contains a compilation of the parameters of erythroid cell proliferation in preleukemia and AML. In preleukemia normal labeling indices (N_s/N_c) as well as normal values of t_s were found for the different morphological cell compartments. These data correspond to the values obtained in a collective of healthy individuals (2). However, the relative production rates (N_s/t_s) show considerable deviation from the normal ratio of 1:2:5 for proerythroblasts:basophilic:polychromatic erythroblasts. The reduction in relative production of more mature erythroblasts most likely is an expression of intramedullary cell death. On the left side of Fig. 1 a scheme of divisions derived from the ratio of production rates illustrates the birth of 3 basophilic erythroblasts from 2 proerythroblasts, and of 2 polychromatic from the 3 basophilic erythroblasts.

The scheme of erythropoietic cell division has not changed much in AML (Fig. 1, right side) as is obvious from the ratio of production rates of 1:1.4:0.9. The rate of cell proliferation, however, is reduced as far as conclusions can be drawn using N_S/N_C and t_S only. Similar findings have been reported in other bone marrow infiltrating diseases (2).

Preleukemia

	MB	PM	MC
N_C (rel.)	723	77	200
N_S/N_C	0.06	0.47	0.25
t_S (h)	15.6	12.8	17.2
N_S/t_S (rel.)	1.8	2.0	2.0

AML

	MB	PM	MC
N_C (rel.)	889	39	72
N_S/N_C	0.07	0.20	0.15
t_S (h)	18.1	15.8	17.9
N_S/t_S (rel.)	14.4	2.0	2.4

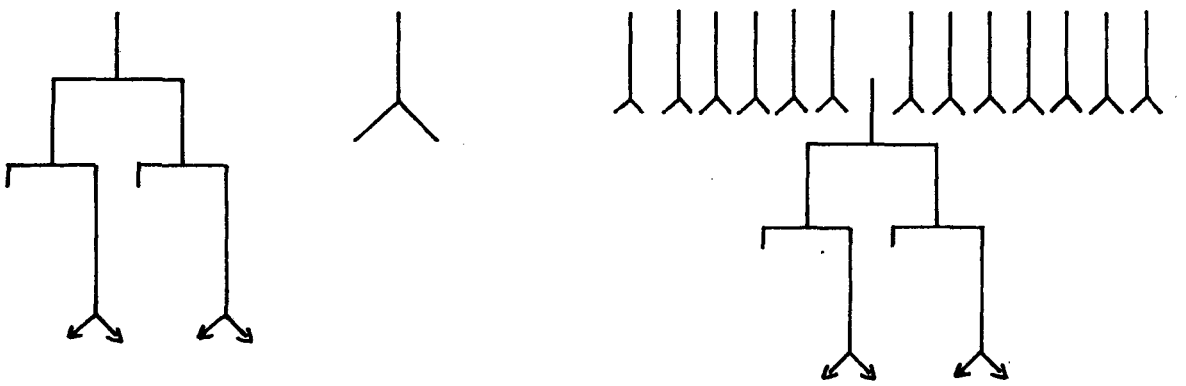

Fig. 2: Parameters of cell kinetics and schemes of divisions of myeloid cells in preleukemia (left side) and AML (right side). Abbreviations: MB = myeloblasts; PM = promyelocytes; MC = myelocytes. For the other abbreviations, see legend to Fig. 1.

In the myeloid series (Fig. 2) a prolonged t_S is found already in preleukemic myeloblasts and myelocytes. Normal values for these cells obtained with the same method have been reported by Brinkmann and Dörmer (1). The labeling index of myeloblast in preleukemia is as low as in AML. In this latter stage t_S ist prolonged in all myeloid compartments. From the production rate in AML amounting to 14:2:2 a high degree of self-renewal of the myeloblast compartment can be deduced. In addition, there may be some ineffective myelopoiesis at the stage of myelocytes which is also observed in the preleukemic phase. Possibly the production rates in preleukemia already indicate that half of the myeloblasts do not give rise to promyelocytes after division but remain myeloblasts.

The various parameters of cell proliferation in preleukemia, especially the high percentage of 94 % of myeloblasts in phases other than DNA synthesis, suggest that these cells already constitute a leukemic population. Table 1 shows that the production rate of this population is much lower than that of erythroblasts at the same stage. In normal bone marrow the ratio of production rates of myeloblasts: proerythroblasts is in the order of 1:1 (3). On the other hand, in AML there is a six-fold increase of the myeloblast production rate over that of proerythroblasts.

Table I: Relative Production Rates (Cells Produced per Unit of Time per 100 Proerythroblasts) in Bone Marrow of Preleukemia and AML

	Preleukemia	AML
Myeloblasts	11	640
Promyelocytes	12	89
Myelocytes	12	106
Proerythroblasts	100	100
Basophilic erythroblasts	130	141
Polychromatic erythroblasts	70	88

The findings in this investigation raise one cardinal question: How can myeloblasts in preleukemia characterized by a reduced proliferative activity as well as a very low production rate overgrow the other cell types and attain such a high rate of new cell formation in AML? The most plausible explanation depends on the assumption of a change in the mode of proliferation. By this change is meant a transition from steady state growth of myeloblasts to some kind of exponential expansion. Under steady state conditions a compartment is being replaced by exactly the same number of cells which are leaving it. In exponential growth some of the daughter cells do not leave the compartment and remain mitotable. This increases the production rate of the compartment even if the individual cell looses some of its proliferative activity. In overt AML, finally, the myeloblast compartment has grown to such a size that it can be regarded as mainly self-maintaining. From the present study there is no answer to the question whether at all or to what extent such a compartment is dependent on the influx of stem cells. However, it is most likely that the rate of cell birth in the compartment exceeds by far the rate of influx into it.

Literature

1. Brinkmann, W. and P. Dörmer: Proliferationskinetik der normalen Myelopoese des Menschen, in Stacher, A. and P. Höcker (eds.): Erkrankungen der Myelopoese. München–Wien, Urban & Schwarzenberg 1976.
2. Dörmer, P.: Kinetics of erythropoietic cell proliferation in normal and anemic man. A new approach using quantitative ^{14}C-autoradiography. Progr. Histochem. Cytochem, vol. 6, no. 1, G. Fischer, Stuttgart 1973.
3. Dörmer, P. and W. Brinkmann: A new approach to determine cellcycle parameters in human leukemia, in T. M. Fliedner and S. Perry (eds.): Prognostic factors in human acute leukemia. Oxford, Pergamon Press 1975.