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Proliferation and Maturation of Hemopoietic Cells in Adult Patients with Different Forms of Acute Leukemia and Chronic Myeloid Leukemia in Agar and Liquid Cultures

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The cloning of haemopoietic cells in semi-solid agar medium makes it possible to evaluate their response to the colony-stimulating factor (CSF). The response to CSF suggests that target cells are of myeloid origin. However, the lack of response to CSF does not exclude the possibility of myeloid origin of target cells, since the blast cells of some patients with acute myeloid leukaemia (AML) do not respond to CSF in agar culture. The cultivation of the haemopoietic cells in liquid cultures (Golde and Cline 1973) makes it possible to investigate the maturation of blast cells, and the origin of blast cells can be determined by the analysis of morphologically distinguishable daughter cells.

A. Material and Methods

A total of 183 patients were investiged (Table 1). The morphological type of blast crisis (BC) and acute leukemia (AL) was identified using common morphological and cytochemical methods (Giemsa, Sudan black, myeloperoxidase, PAS). Janossy revealed a good correlation between cytomorphological and immunological division of BC into "myeloid" and "lymphoid" types (M- and L-type).

The cloning of hemopoietic cells was performed in the double layer agar culture by Pike and Robinson (1970) with slight modification (Afanasiev et al. 1976). Colonies (>20 cells) and clusters (3–20 cells) were scored at day 6–7. It was possible to characterize four types of growth patterns in bone marrow cell cultures: hypoplastic, normal, hyperplastic and leukaemic (Fig. 1).

In patients with AL the response of haemopoietic cells to CSF was designated when the leukaemic type of growth was seen in agar culture and in patients with CML when leukaemic or hyperplastic growth was seen. The maturation of blood and/or bone marrow blast cells (acute lymphoblastic leukaemia [ALL] – 6, acute non-lymphoblastic leukaemia [ANLL] – 16, acute undifferentiated leukaemia [AUL] – 4, and CML-BC – 11 patients) was studied in the liquid culture system by Golde and Cline. Morphological investigation was performed at 7–14 days.

Table 1. Patients studied

Diagnosis	Number of patients
Acute lymphoblastic leukaemia (ALL)	31
Acute non-lymphoblastic leukaemia (ANLL)	45
Acute undifferentiated leukaemia (AUL)	10
Haemopoietic dysplasia (HD) (Smouldering leukaemia)	9
Chronic myeloid leukaemia-blast crisis (CML-BC)	26
Chronic myeloid leukaemia-chronic stage (CML-CS)	43
Chronic myeloid leukaemia-accelerated stage (CML-AS)	16
Myelofibrosis-blast crisis (MMM-BC)	3
Control group	72

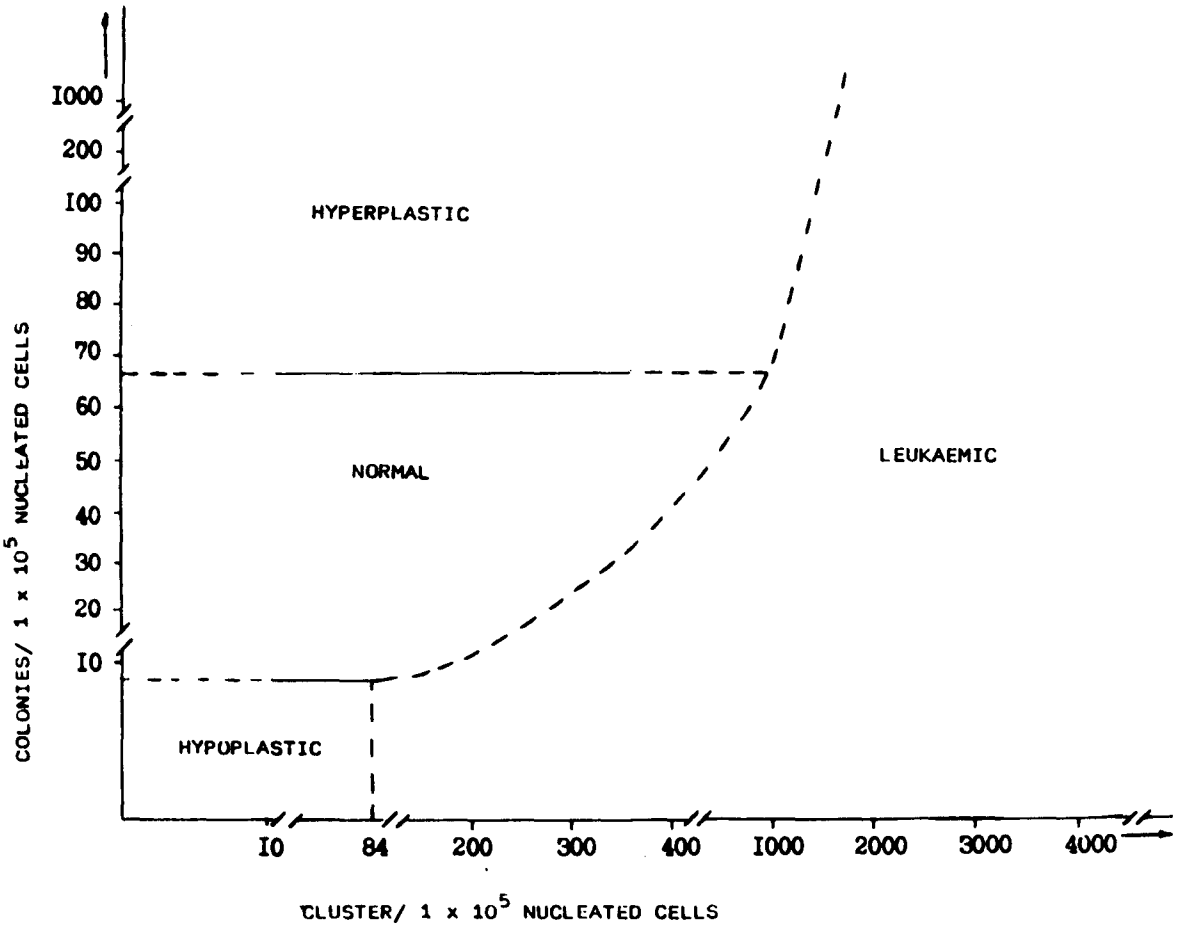

Fig. 1. Picture of zones from different growth patterns of bone marrow cells and the criteria for definition of growth types of bone marrow cells in double layer agar system. *normal,* colony count from 8 to 67, normal size of colonies, cluster: colony<12; *hypoplastic,* colony count<8, and cluster count<84; *hyperplastic,* colony count>67, and cluster: colony<12; *leukaemic,* cluster: colony>12, small size of colonies (20–40 cells) and consisting of single cells

B. Results

The results of the study of haemopoietic cell growth type in patients with AL in agar cultures are presented in Fig. 2. The leukaemic type of growth (response of leukaemic cells to CSF; a myeloid cell property) has been observed in 6% of ALL, 67% of ANLL, 50% of AUL and 56% of haemopoietic dysplasia (smouldering leukaemia). In 6 of 16 patients with ANLL (non-maturation group) the blast cells had an absolute arrest of maturation at the level of blasts-promyelocytes and blasts-promonocytes in liquid culture. In ten patients (maturation group) the blast cells possessed the ability to mature to monocytes-macrophages and/or immature granulocytes. In patients of this group the ability of blast cells to mature was more pronounced in the monocytes-macrophages cell line than in granulocytes. In patients with AUL and ALL we have observed an intensive granulomonocytopoiesis in a 7-day-old liquid culture. The results of the study of the growth type of haemopoietic cells in patients with different stages of CML are presented in Fig. 3. In 75% of the patients with the L-type of CML-BC and 82% with the M-type haemopoietic cells responded to CSF in double layer agar culture. In most of the patients with both L-type (five of seven) and M-type (three of four) of blast crisis the blast cells possessed the ability to mature to granulocytic and monocytic cells in the liquid culture system. This finding has been observed in two patients with both the L-type of CML-BC and neuroleukaemia.

C. Discussion

The results obtained confirm the data that the patients with AL are a very different group in

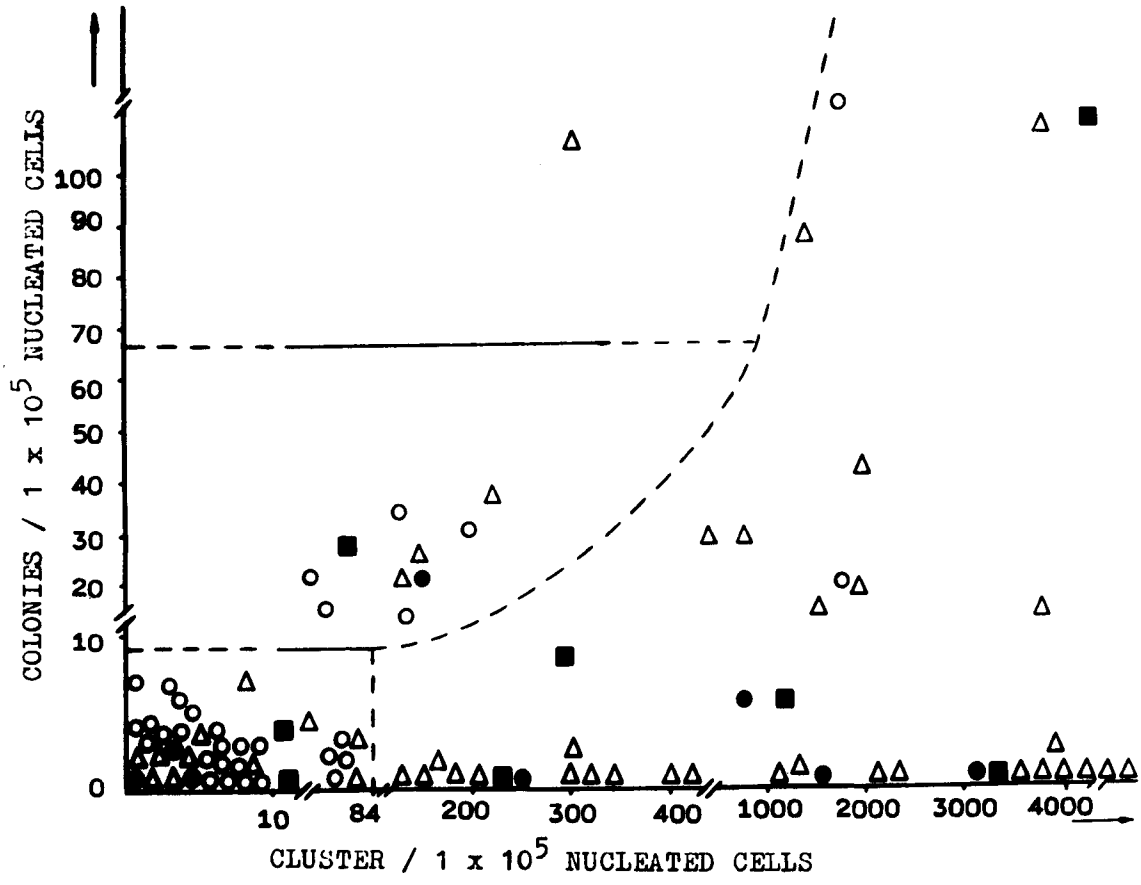

Fig. 2. Growth patterns of bone marrow cells in patients with different forms of acute leukemia (double layer agar technique). ○, ALL; △, ANLL; ●, AUL; and ■, HD

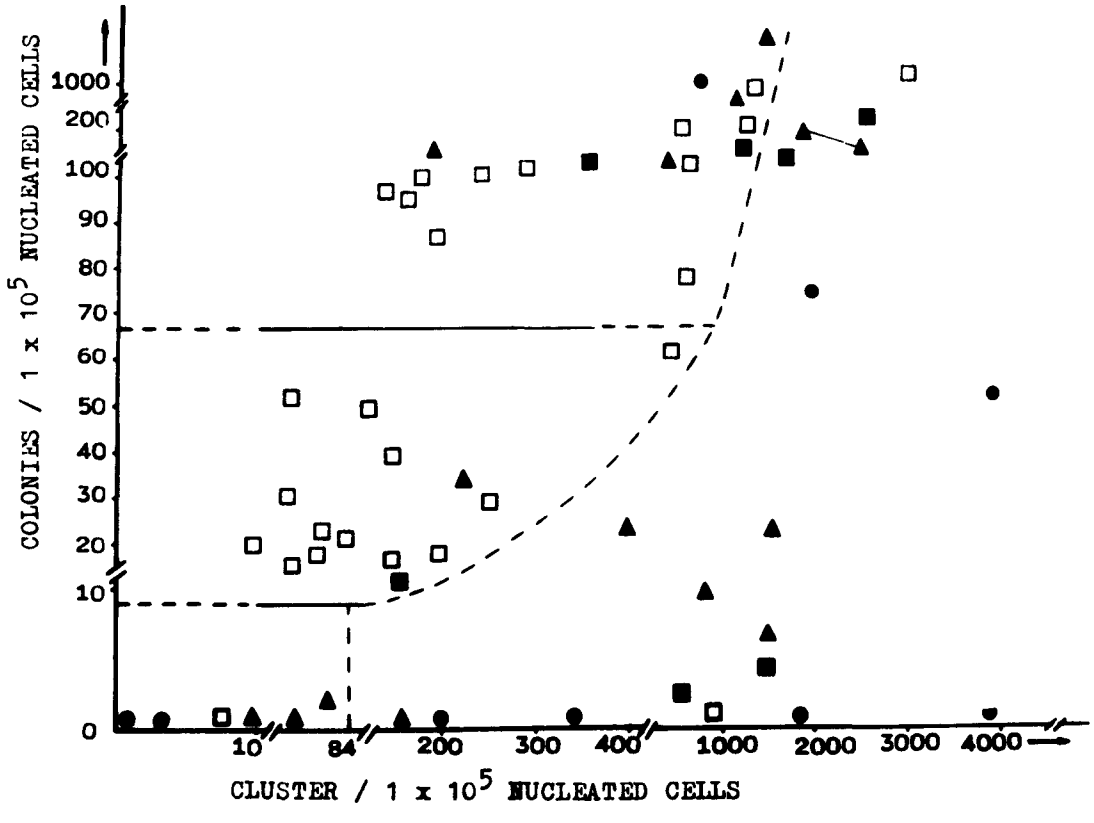

Fig. 3. Growth patterns of bone marrow cells in patients with CML (double layer agar technique). □ CML (chronic stage), ■ CML (accelerated stage), ● CML (blast crisis, "lymphoid type"), and ▲ CML (blast crisis, "myeloid type')

their haemopoietic cells' ability to proliferate and mature in vitro. In ANLL patients haemopoetic cells responded to CSF much more frequently than in ALL. Only in 6% of ALL patients there was a typical leukaemic type of growth patterns in agar culture. These data are in agreement with Spitzer et al. (1976) who have discovered a leukaemic type of growth pattern in agar culture of haemopoietic cells in some patients with AL. The origin of these clusters and colonies in agar culture has not been determined. The leukaemic type of growth pattern observed in the agar cultures in 56% of the haemopoietic dysplasia (HD) patients indicates that most of the cases could be considered as patients with true leukaemia.

Our results of cultivating in the liquid culture system indicate that in most of the ANLL patients the ability of blast cells to mature was more pronouced in the monocytes-macrophages cells line than in granulocytes. Cultivation of haemopoietic cells in patients with CML-BC in liquid and agar culture suggests that the type of blast cells is not always determinable in the light microscope by morphological and cytochemical tests. Most of our patients with L-type of BC have blast cells of myeloid origin (response to CSF and ability to mature to granulomonocytic cells). These data are in agreement with Marie et al. (1979) who have discovered that in 9 of 12 patients with BC the blasts contained peroxidase activity detectable only by electron microscope.

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