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Proliferation and Maturation of Hemopoietic Cells from Patients with Preleukemia and Aplastic Anemia in Agar and Diffusion Chamber Cultures

E. Elstner, E. Schulze, R. Ihle, M. Schütt, and H. Stobbe

Aplastic anemia (AA) and hemopoietic dysplasia (HD) (preleukemic syndrome) are diseases which are caused by a defect in the level of hemopoietic stem cells [4, 7, 9, 12] and may result in an acute nonlymphocytic leukemia (ANLL) after a valuable interval. Traditional diagnostic criteria are often insufficient for precise characterization of the above disorders. The present communication aims to illustrate the value of modern proliferation tests (agar colony technique and diffusion chamber technique) for the differential diagnosis and prognostic evaluation of these hemopoietic disorders.

A. Material and Methods

Bone marrow cells of 13 patients with idiopathic acquired AA and eight patients with HD were studied in agar cultures. Diagnostic criteria for HD are presented in Table 1. Bone marrow cells of five patients with idiopathic acquired AA and six patients with HD were examined in diffusion chamber cultures. Bone marrow for controls was obtained from patients without hematological diseases. The cloning of hemopoietic cells was performed in the double-layer agar system described by Pike and Robinson [8]. Colonies (> 40 cells) and clusters (3–40 cells) were scored at day 6–7. For the morphological studies, colonies and clusters were removed from the agar and stained with aceto-orcein.

The other method used for our investigations was the diffusion chamber (DC) technique [1, 3]. The proliferation of human bone marrow cells in DC cultures was estimated on the basis of myelopoiesis occurring in this culture system (proliferation index = I_{prol}).

$$I_{prol} = \frac{\text{number of harvested peroxidase positive cells at day 14}}{\text{number of inoculated peroxidase negative cells at day 0}}$$

(Peroxidase negative cells are known to contain the stem cells). For the morphological investigation smears were stained with May-Grünwald and Giemsa. The maturation of the hemopoietic cells was evaluated by the maturation index (I_{mat}).

$$I_{mat} = \frac{\text{amount of nonproliferative granulopoietic cells at day 14}}{\text{amount of proliferative granulopoietic cells at day 14}}$$

Cells were classified as proliferative granulopoietic cells (blast cells, promyelocytes, and myelocytes) or nonproliferative granulopoietic cells (metamyelocytes, juveniles, bands, and segments).

B. Results

I. Agar Culture

Figure 1 gives the growth pattern of bone marrow from patients with AA and HD in agar culture. The following observations were made:

1. The number of colonies was decreased in both groups of patients (Fig. 1 a). But the

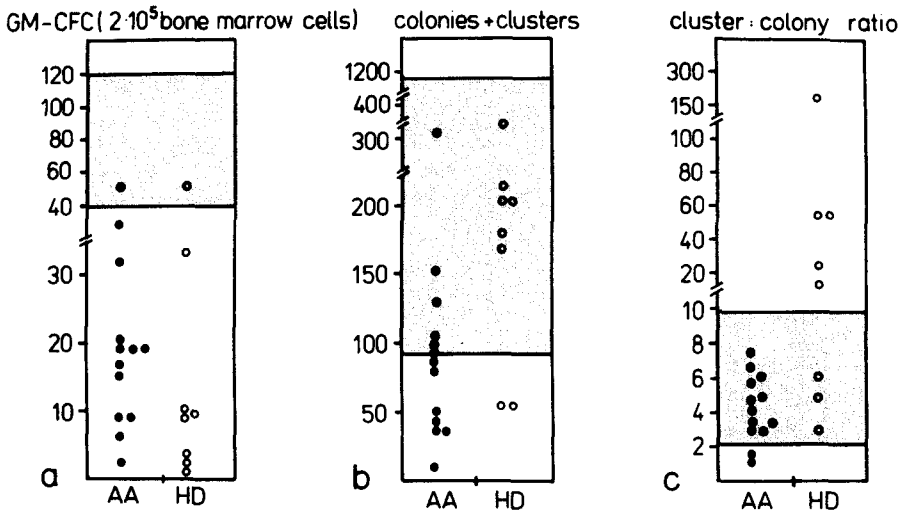

Fig. 1 a–c. Growth pattern of bone marrow from patients with AA and HD in agar cultures. **a** Number of GM-CFC. **b** Total count of aggregates (colonies + clusters). **c** Cluster to colony ratio. *Shaded area,* range of 20 normal volunteers

number of total aggregates was decreased only in patients with AA; in HD patients in most cases it was normal (Fig. 1 b).

2. In AA patients the cluster-to-colony ratio was normal. In contrast, in patients with HD the cluster-to-colony ratio was increased in most cases (leukemic type of growth) (Fig. 1 c).

3. The differentiation of the cells in colonies and clusters was normal in patients with AA, but in HD patients with leukemic type of growth the cells of the clusters were very uniform and of a blast cell type.

II. DC Culture

The results of the behavior of the bone marrow cells in DC culture are presented in Figs. 2–4. The following is obvious:

1. The DC proliferation index (I_{prol}) was lowered in patients with AA, but was not decreased in most cases of HD (Fig. 2 a).

Table 1. The preleukemic syndrome: diagnostic criteria

1. Pancytopenia
2. Hyperplastic bone marrow, less than 5% blasts
3. Megaloblastic or/and sideroblastic erythropoiesis with qualitative deviations
4. Either abnormal megakaryocytes or disorderly granulopoiesis (pseudopelger and others)
5. Absence of B_{12} or folate deficiency
6. No treatment with cytotoxic agents in the 3 months preceding diagnosis

2. The DC maturation index (I_{mat}) was not decreased in AA patients; in contrast, it was lowered in all patients with HD (Fig. 2 b).

When comparing the clinical course with the growth pattern in agar and DC cultures we observe the following:

1. The lowest proliferation rates in both assay systems were found for bone marrow from AA patients with a bad course of these diseases.

2. One patient with an extremely high cluster-to-colony ratio in agar culture and low I_{mat} in DC culture developed ANLL 10 months after the investigation.

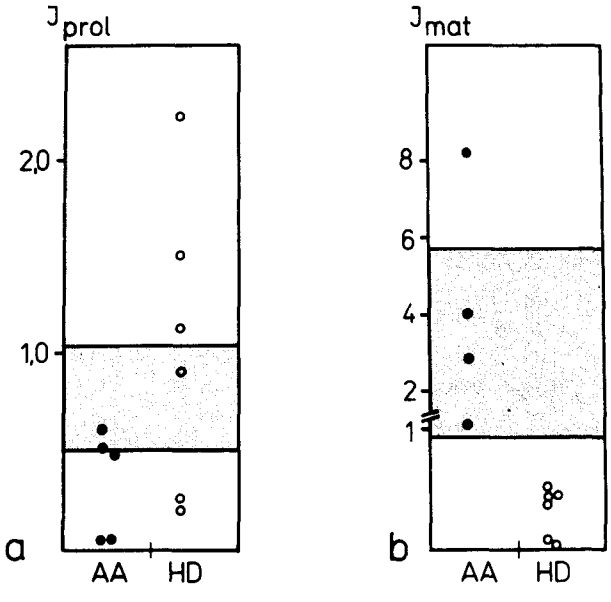

Fig. 2 a, b. Growth pattern of bone marrow cells from patients with AA and HD in diffusion chamber cultures. **a** DC-proliferation index (I_{prol}). **b** DC-maturation index (I_{mat}). *Shaded area* range of four normal volunteers

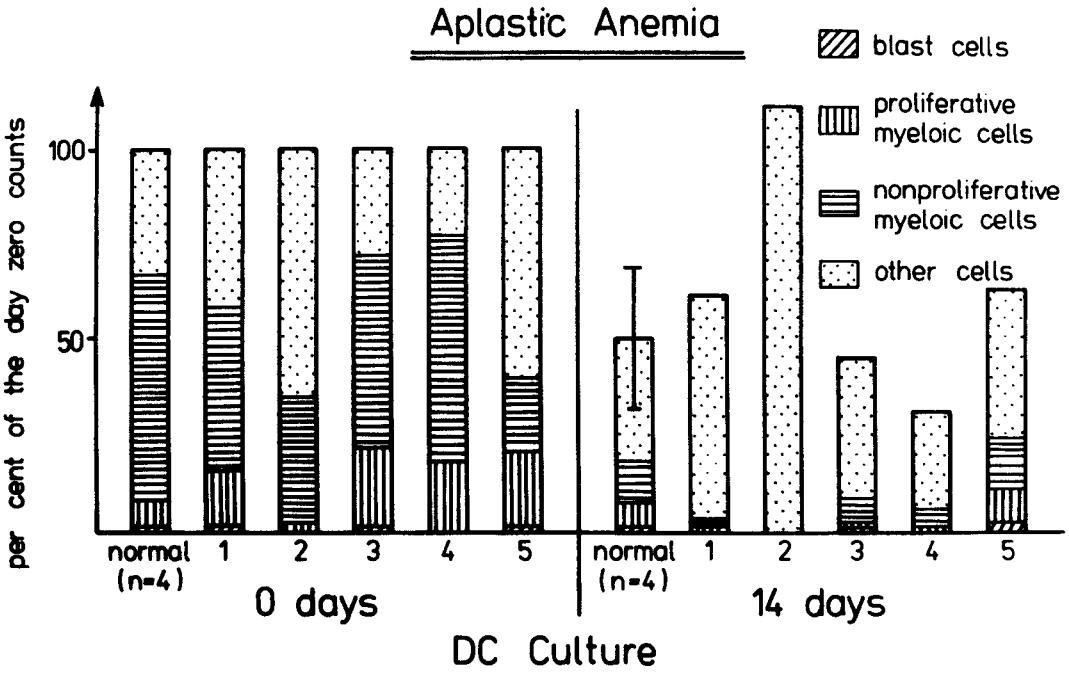

Fig. 3. Differential counts of the day 0 and day 14 diffusion chamber contents

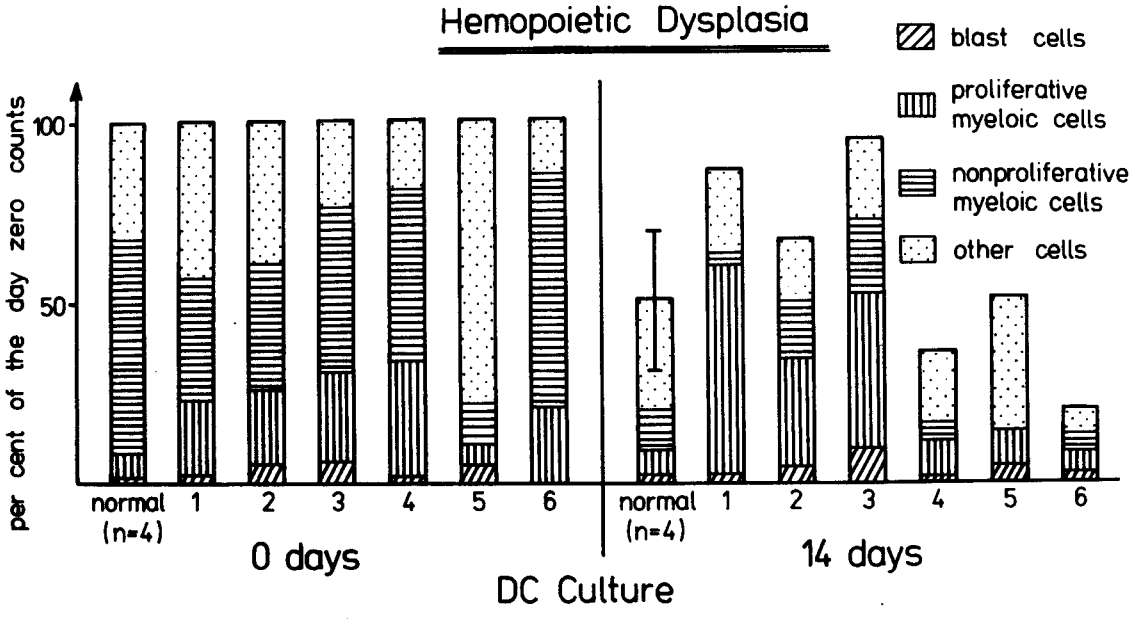

Fig. 4. Differential counts of the day 0 and day 14 diffusion chamber contents

C. Discussion

Precise characterization of AA and HD by means of the traditional diagnostic criteria is often difficult. Our results (Table 2) show that there are distinct differences in the growth behavior of the hemopoietic cells in patients with these disorders. The results of both culture systems point to a true proliferation decrease in the bone marrow of patients with AA: the number of GM-CFC and of total aggregates in agar and the DC-proliferation index were lowered. These findings are consistent with those of other authors, who have noticed a diminished stem cell compartment in AA [2, 5, 6, 10]. The maturation, in contrast, was unimpaired in both systems in this group of patients.

Bone marrow cells from our patients with HD demonstrated an abnormal proliferation in agar culture. This means the

Table 2. Growth pattern in agar and DC culture from patients with AA and HD

	AA	HD
Agar culture		
Number of colonies	↓	↓
Number of total aggregates	↓	Normal
Cluster to colony ratio	Normal	↑
DC culture		
Proliferation index	↓	Normal ↑
Maturation index	Normal	↓

number of colonies was decreased and the cluster-to-colony ratio was markedly increased (Table 2). This in vitro growth pattern is similar to those seen in patients with ANLL. In contrast to the decreased number of colonies the total number of aggregates in agar and the DC-proliferation index were not diminished. Therefore, the reduced number of colonies in the agar culture suggested no proliferation decrease in HD patients, but the establishment of a cell clone with a defective responsibility to CSA in agar [11]. Furthermore, the maturation ability of the hemopoietic cells in HD was strongly affected in both assay systems.

Our results corresponded to the clinical course of the diseases in both systems: the lowest proliferation rates were found for bone marrow of AA patients with a bad course of these diseases; one HD patient with an extremely high cluster-to-colony ratio and a low DC-maturation index developed ANLL 10 months after the investigation.

These observations indicate that growth patterns and maturation ability of bone marrow cells in agar cultures and DC may add to the traditional morphological and clinical criteria for differential diagnosis and prognostic evaluation of these hemopoietic disorders.

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