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Human Bone Marrow Granulocyte-Macrophageal Colonies (GM-Cs) *in vitro*: Morphological/Rheological Features of Myeloid Cells in Health and in Cases of Myeloid Leukemia

E. Zvetkova, Y. Savov , Y Gluhcheva, I. Ilieva, E. Bichkidjieva, E. Katzarova, I. Tsenov

Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Sciences, Sofia

The morphological characteristics of granulocyte/macrophageal (GM-) colonies and clusters, obtained *in vitro* (in semi-solid agar cultures) from bone marrow hematopoietic myeloid progenitors pertain to leucocyte hemorheology of healthy persons and patients with myeloid leukemias. The morphological features of *in vitro* growing myeloid progenitors, granulocytes and macrophages of healthy persons differ in their cell size, shape and degree of differentiation from the cultivated marrow cells in cases of acute and chronic myeloid leukaemia. In this malignant disease, the rheological properties of leucocytes (granulocytes/ macrophages) were found to provide diagnostic information. Further studies should be undertaken to examine whether the method could be useful in defining survival, prognosis and therapeutical approach in cases of myeloid leukemia.

Key words: human bone marrow agar cultures; granulocyte/macrophageal (GM-) colonies and clusters *in vitro*; morphological/rheological features of myeloid cells; healthy persons, myeloid leukemia patients.

Introduction

Bone marrow hematopoietic and stromal cell progenitors give rise to marrow and stroma cell growth and differentiation under the influence of haematopoietic growth factors (colony-stimulating factors – CSFs, cytokines) [5]. The diagnostics methods for determination of hemathological paramethers of marrow cells - in health and hemathological diseases, do not feature the morphological/rheological changes of the *in vitro* cultivated myeloid cells and their precursors.

The aim of the present study is to examine the morphological/rheological characteristics of cultivated *in vitro* (in conditions of semi-solid agar cultures) marrow cells from the granulocyte-macrophageal (GM-, myeloid) lineage of healthy persons and patients with acute and chronic myeloid leukemias (AML, CML).

Material and Methods

Human bone marrow samples (derived from 5 healthy donors and 6 patients with acute and chronic myeloid leukemias) were examined *in vitro* – in conditions of marrow semi-solid (agar) cultures, prepared by the method of M e t c a l f et al. [3, 4]. The granulocytes and macrophages (G-, M-) from hemopoietic colonies and clusters, obtained in agar cultures at the 7th day of cultivation, were stained *in situ* with methylene blue and fast green (after the cytochemical method of Z v e t k o v a and J e l i n e k [8] for DNP, RNP and some basic (cationic) proteins and were examined light microscopically. The mature cells of the granulocyte series were identified by their typical cell size, nuclear shape and the green coloured cytoplasmic granules containing basic proteins, while the ones of macrophageal origin were characterized by their metachromatic cytoplasmic granules (Fig. 1, 2).

Results and Discussion

The morphological features of *in vitro* growing myeloid progenitors, granulocytes and macrophages of healthy persons differ in their cell size, shape, cytoplasmic granules and degree of differentiation from the cultivated marrow cells in cases of acute and chronic myeloid leukaemia.

The myeloid cell colonies and clusters obtained in marrow agar cultures of healthy people consist of the macrophageal-granulocyte or purely macrophageal cell types with predominance of fully differentiated cells in them (Fig. 1, 2). Some colonies and clusters of blast-like cells (with compact and/or ring-shaped nucleoli, dispersed homogeneous nuclear chromatin and diffusely bluish stained cytoplasmic RNP) could be also visualized (Fig. 3). All these myeloid cells and precursors differ in their degree of differentiation and morphological/rheological features from the atypical and pathologically immature cells in marrow agar cultures from myeloid leukemia patients (Fig. 4). Simultaneously, the number of mature GM-colonies and clusters is reduced or they are not presented in agar cultures of ML-patients - probably in relation to the deficiency of the GM-maturation and granulopoietic response in cases of ML, perhaps acting as a feedback signal and leading to a greater proportion of atypical myeloid blast cells involved in cycle [1]. Each of these atypical GM-precursors differs in its cell shape, size and cytoplasmic granules, compared to other myeloid precursors and insufficiently differentiated cells in the same cell group (Fig. 4) as well as to the late forms of neutrophils (methamyelocytes and bands) and mature monocytes/macrophages in the marrow colonies and clusters of healthy persons (presented in Fig. 1,2). Furthermore, since normally the less committed progenitor cells (at least bipotential) undergo amplification to generate granulocytes and monocytes/macrophages and represent myeloid progenitors with a colony-forming capacity, the myeloid progenitors in the ML-cases are blocked in their differentiation and they display a significantly reduced pool of mature GM-Cs in the marrow agar cultures of myeloid leukemia patients.

The phenomenon was most striking in the blast cell population indicating that the blast cell cycle is faster in ML-patients. The pathologically increased blast cell cycling in the marrow of leukemia patients promotes in vitro production of the increased number of cell clusters containing atypical myeloid precursors: blasts and blast-like cells, immature neutrophils (neutrophil precursors at the promyelocyte and myelocyte stage) and atypical immature monocytes/macrophages (Fig. 4).

It is a well known fact that the release of mature neutrophils and monocytes from the bone marrow of ML-patients is impaired while they demonstrate markedly de-

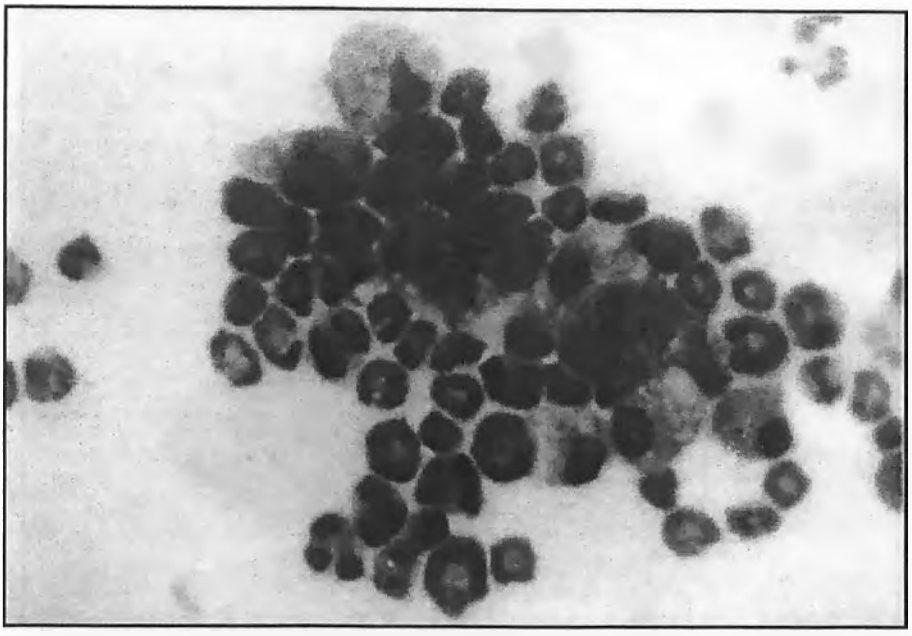

Fig. 1. Granulocyte-macrophageal (GM-) colony in bone marrow agar culture of healthy person – with predominance of fully differentiated granulocytes and macrophages. Immersion × 1000

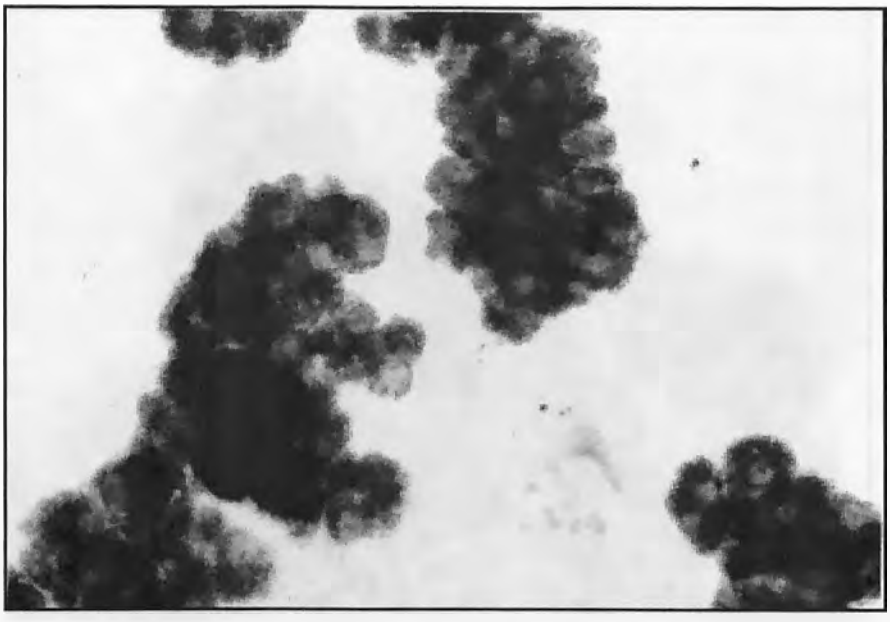

Fig. 2. Macrophageal colonies in bone marrow agar culture of healthy person: methachromatically stained agar particles in the cytoplasms of phagocyting cells can be observed. Immersion × 850

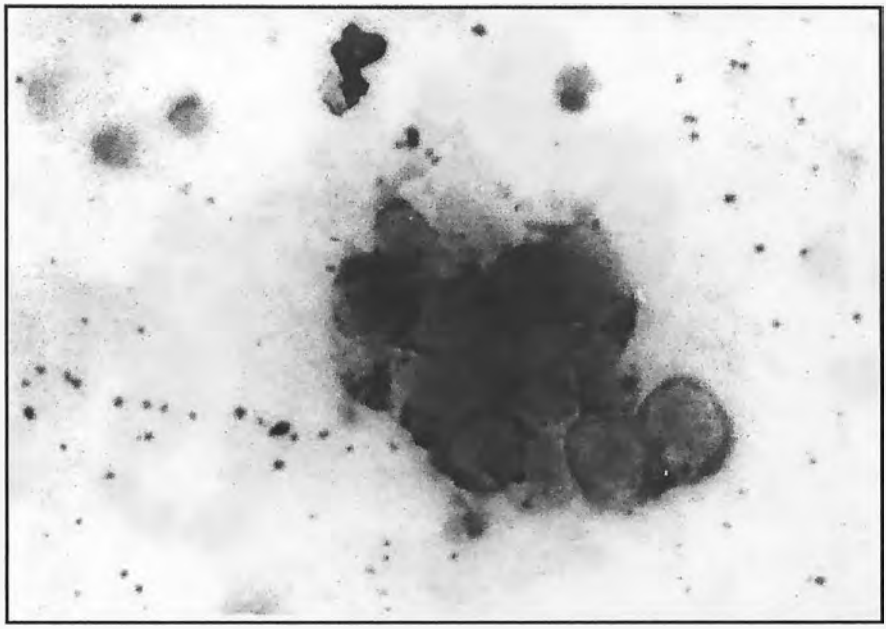

Fig. 3. Colony of undifferentiated blast-like cells in bone marrow agar culture of healthy person. Immersion × 1250

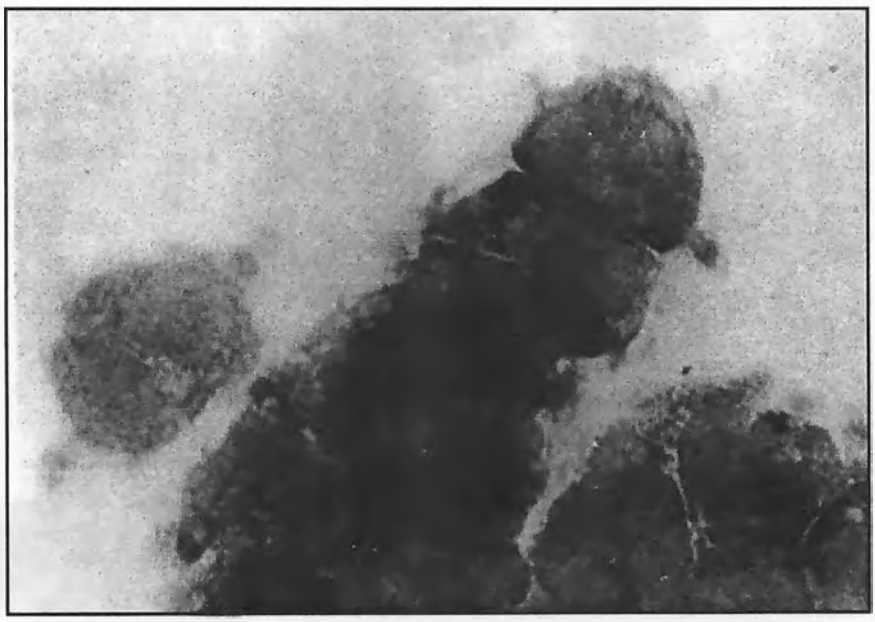

Fig. 4. Cluster of atypical blasts and pathologically immature myeloid cells in bone marrow agar cultures from chronic myeloid leukemia (ML-) patients. Immersion × 1450

creased hemotaxis in response to IL-8 [2, 6]. Thus, some kind of signalling by certain hematopoietic factors is probably required for GM-morphologic differentiation, rheological properties or lineage commitment [2]. These signals appear to be important for the in vivo and in vitro neutrophil/monocyte differentiation, functional phenotype and biological activities – including phagocytosis, alterations in hemotaxis and the adhesive interactions between myeloid and stromal cells in the bone marrow as well as for the subsequent transmigration of granulocytes through the endothelial cell layer and the subendothelial basal lamina.

The results from our and other studies [1, 2, 6, 7, 8] suggested that various biologically active substances have been implicated to play a role in the mobilization of normal and/or pathological myeloid cells and their precursors from the bone marrow to the blood and could be important for the leucocyte hemotaxis, migration, adherence and other rheological properties – in response to mobilizing stimuli as cytotoxic agents, chemokines (IL-8), haematopoietic growth factors, etc.

Conclusion

The methods for determination of marrow hemathological parameters of healthy people and myeloid leukemia patients could include morphological/rheological *in vitro* analysis of granulocyte/macrophageal colonies (GM-Cs) and clusters as an additional technique in the laboratory and clinical diagnostics.

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