Institute of Experimental Morphology and Anthropology with Museum

Acta morphologica et anthropologica, 11
Sofia • 2006

The Influence of Interferon-gamma on the Processes of Proliferation and Differentiation of Hematopoietic CD34+ Progenitor Cells in Human Erythropoiesis

Y. Gluhcheva, K. Schroecksnadel, B. Wirleitner*, I. Ilieva, E. Bichkidjieva, B. Nikolov, E. Zvetkova, G. Konwalinka**, D. Fuchs**

Institute of Experimental Morphology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia
**Institute for Medical Chemistry and Biochemistry, Innsbruck, Austria*
***Department of Internal Medicine, Medical University, Innsbruck, Austria*

Interferon-gamma (IFN-γ) stimulates erythroid colony formation when added to cultured in vitro CD34+ human hematopoietic progenitor cells in the presence of Recombinant cocktail of factors (including SCF and IL-3) or Agar-leucocyte conditioned medium. Stronger stimulatory effect of the cytokine was observed for the BFU-E compared to the CFU-E colonies (possibly due to the different degrees of maturity of the progenitor cells that give rise to the two types of erythroid colonies). When investigating the surface of the cells within the erythroid colonies using scanning electron microscopy (SEM), structures reminding of pseudopodes were observed.

Key words: CD34+ progenitor cells, erythropoiesis, erythroid colonies, INF-γ, scanning electron microscopy – SEM.

Part of this work was presented on the Sixth International Symposium of Clinical Anatomy, held in Varna, October 8–10, 2004.

Introduction

Erythropoiesis is the process of formation of mature red blood cells from immature bone marrow progenitors. CD34+ cell subset is the most frequently used cell population for in vitro assays and a unique one capable of self-renewal and differentiation into mature erythroid cells. While cultured in vitro these cells form two types of colonies: colony-forming unit-erythroid (CFU-E) and burst-forming unit-erythroid (BFU-E). They differ in the degree of maturity of the progenitor cells that initiate their formation. It is known that the CFU-E colonies are formed from more differentiated cells than those in the BFU-E colonies. For this reason the appearance of CFU-E colonies is observed in vitro (in semi-solid agar cultures) earlier than the BFU-E.

In order to proliferate and differentiate the erythroid progenitor cells require growth factors such as stem cell factor (SCF), interleukin-3 (IL-3), interleukin-6 (IL-6),

erytropoietin, different colony stimulating factors (CSFs), etc [8]. On the other hand, there are growth factors that inhibit cell proliferation and colony formation or exert bipotent effects. Such factor is interferon-gamma (IFN-γ). There are data [1, 10, 11] showing that when IFN-γ is added to cultured in vitro hematopoietic cells it inhibits the formation of erythroid and mixed (granulocyte/macrophage) colonies. On the other hand, when this factor is added to cells cultured in SCF and IL-3 it synergizes their effects, thus it stimulates progenitor cell proliferation [3, 5, 6]. There are only few sources investigating the cell-to-cell interactions among the CD34+ cells [7] and no such data for the contacts between the cells within the erythroid colonies. We were also interested to observe the surface of the erythroid colonies (by SEM) having in mind that there are not enough data for the morphological/ultrastructural characteristics of the erythroid hematopoietic colonies cultured in soft agar in vitro.

Material and Methods

Cell cultures

Purified and enriched human hematopoietic CD 34+ progenitor cells (kindly provided by Laboratory of Immunology – Innsbruck University) were cultured in semi-solid agar cultures. Two experimental systems were developed:

– cells cultured in recombinant cocktail (RC) which consisted of SCF and IL-3 (Chemicon, Austria);

– the two cell types were also cultured in Agar-Leucocyte Conditioned Medium (Agar-LCM) (CellSystems Biotechnologie Vertrieb GmbH, Austria).

Erythropoietin (Erypo-Janssen-Cilag Pharma, Austria), glutamine and mercaptoethanol were added to both experimental models. To investigate the effects of the different doses of IFN-γ (Rentschler Biotechnologie GmbH & Co.KG, Austria) it was added at 5000 U/ml once; 200 and/or 400 U/ml – every second day. The cell cultures were incubated for 14 days at 37°C in humidified air of 5% CO_2. After incubation the colonies were scored on inverted microscope and stained with May-Grünwald/Giemsa (Merck). To determine the type of erythroid colonies – CFU-E or BFU-E, the following criteria were used [2]:

– CFU-E consist of less that 65 cells and are fully hemoglobinized after 14 days of incubation;

– BFU-E consist of more than 65 cells and form colonies with at least two clusters or bursts. They can /or cannot be fully hemoglobinized after 14 days of incubation.

Scanning Electron Microscopy

The samples for SEM were fixed in 2.5% glutaraldehyde (Merck), mounted on disks and covered with 20–30 nm of gold. The probes were investigated by scanning electron microscope Philips-515. The colonies were observed at different magnifications (varying from 20 to 8000×) and were recorded as microphotographs.

Results

Stimulatory effect of IFN-γ on BFU-E formation was observed in both types of cultures – purified and enriched (Fig.1). This effect is better expressed when the cells were cultured in RC. More BFU-E formed by purified cells were observed when the cytokine was added more frequently but at lower doses (200 and/or 400 U/ml). No significant

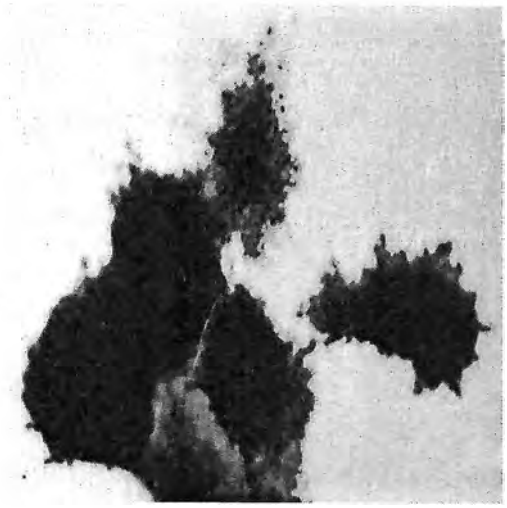

Fig. 1. Erythroid colonies in semi-solid agar medium (× 160)

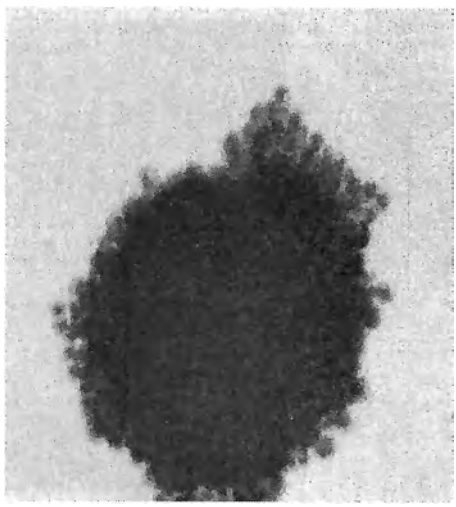

Fig. 2. Immature erythroid colony (× 400)

Fig. 3. Mature erythroid colony (× 400)

differences on the impact of the different doses of IFN-γ were found when purified cells were cultured in Agar-LCM. Weaker stimulatory effect of the cytokine was observed when enriched cells were cultured in the same culture medium. BFU-E are lager in size than the CFU-E and less differentiated – with more basophilic cytoplasm and fine dispersed nuclear chromatin (Fig. 2).

Neither stimulatory nor inhibitory effect of IFN-γ was observed for the CFU-E. This type of colonies consist of more differentiated cells. The erythroid cells located in the periphery of the colonies have piknotic nuclei and eosinophilic cytoplasm – due to the large amounts of hemoglobin (Fig. 3).

The surface of the erythroid colonies was investigated by the method of SEM (Fig. 4a). The surface of the BFU-E (immature erythroid colonies) is smooth and the indi-

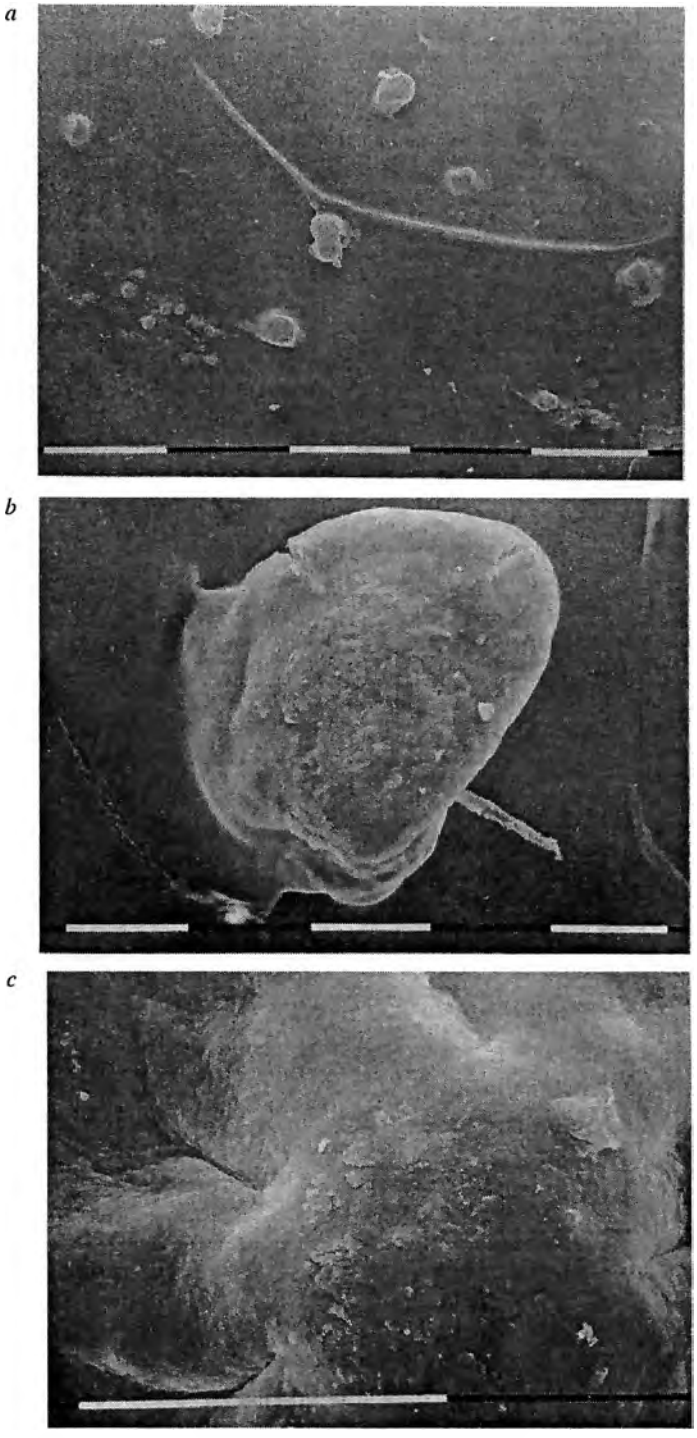

Fig. 4. Electron micrographs of immature erythroid colonies:
a – erythroid colonies in semi-solid agar, observed by SEM (× 32); b, c – surface of immature erythroid colonies (BFU-E) (× 320, × 960)

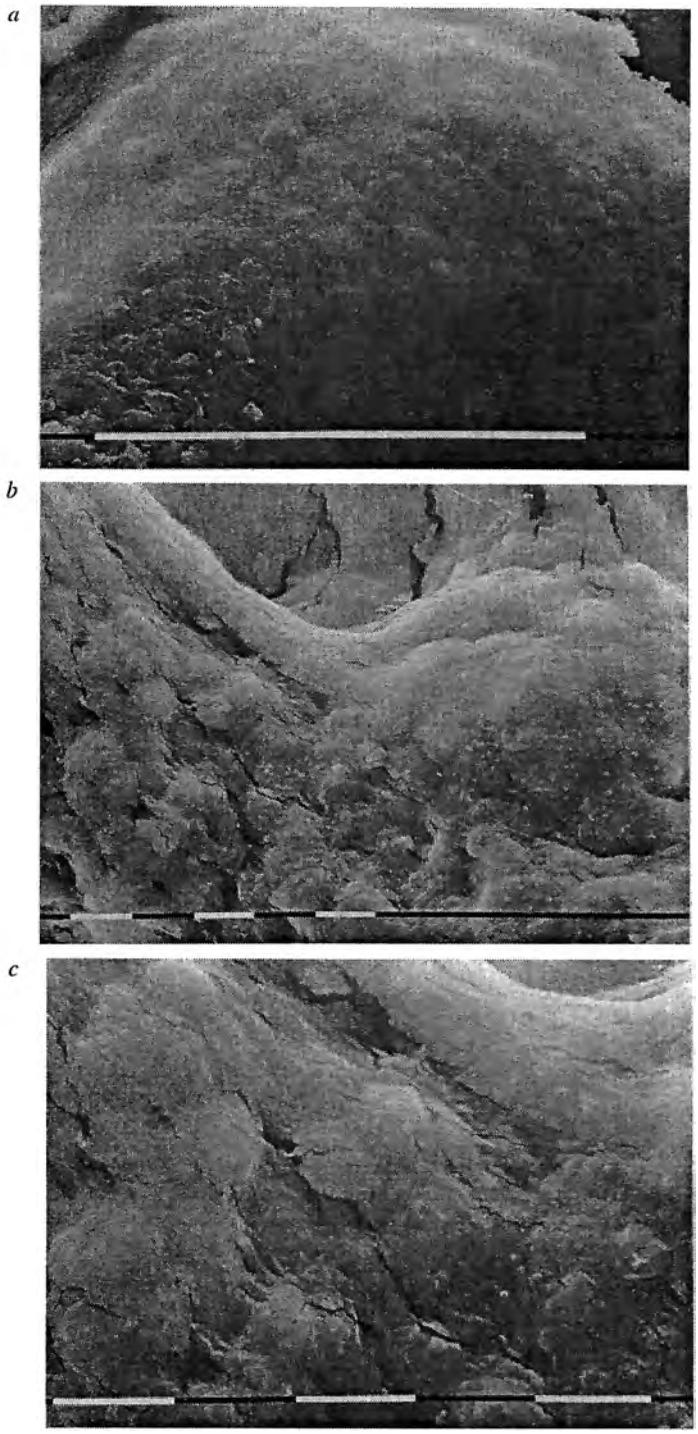

Fig. 5. Electron micrographs of mature erythroid colonies:
a – mature erythroid colony (CFU-E): the outlines of the individual cells can be observed (× 1280); b, c – mature erythroid colonies (CFU-E): cell contacts can be seen (× 1600; × 3200)

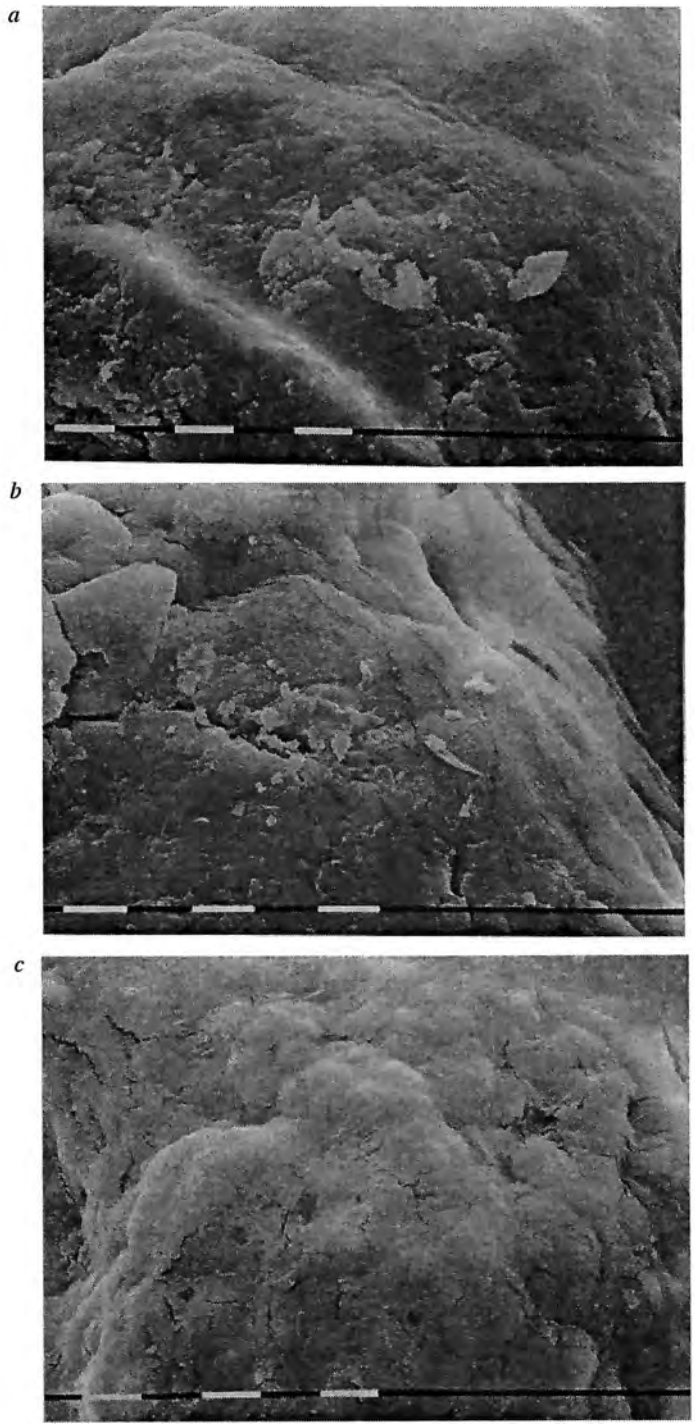

Fig. 6. Extracellular extrusions:
a, b – extracellular extrusions on the surface of a CFU-E (× 1600); c – structures reminding of round pores are observed on a CFU-E; cell-to-cell contacts can be seen (× 1600)

vidual cells cannot be easily distinguished (Fig. 4b, c). On the other hand, on the surface of a CFU-E (mature erythroid colonies) the outlines of the individual cells can be seen (Fig. 5a, b, c). On the surface of both types of colonies (BFU-E and CFU-E) we found specific extracellular structures (extrusions – Fig. 6a, b) which were probably extruded from the cell's cytoplasm. On microphotographs the CFU-E reminds of a "budding yeast"; using higher magnification we also observed superficial *structures* that look like *round pores* (Fig. 6c). Cell-to-cell contacts were also registered (Fig. 5b, c, 6c).

Discussion

The effect of IFN-γ on colony formation of hematopoietic progenitor cells in vitro depends on the dose and duration of IFN-γ influence, the other growth factors present in the culturing medium and the stage of cell maturity.

Our results are in contrast with those previously reported by A l l e n et al., C o u t i h n o et al., W a n g et al. [1, 4, 11], which indicate that the "... inhibitory effect is most marked in the more primitive cells than the committed colony– and cluster-forming cells". On the other hand, we also observed almost no effect of IFN-γ on the colony-forming units. This is possibly due to the higher degree of maturity of the erythroid progenitor cells which makes them more resistant or less sensitive to the influence of the inflammatory cytokine. The results obtained are in agreement with those of Shiohara et al. [9] which indicate that IFN-γ – in combination with SCF, stimulated the development of primitive hematopoietic progenitors as well as the development of mature cell populations. The authors hypothesize that SCF supports the early stages of erythroid progenitor cell proliferation and require IFN-γ for subsequent growth and differentiation.

Our investigation of the erythroid cell surface within the hematopoietic colonies by SEM is the first in the scientific literature since there are data dealing only with isolated individual progenitor cells [7]. In regard with these and our results we hypothesize that the extracellular structures (extrusions), observed on the erythroid cell surface in both types of colonies – BFU-E and CFU-E, could be products formed in the coarse of cell differentiation and colony development.

Conclusion

IFN-γ stimulated the processes of erythroid proliferation and differentiation of the hematopoietic CD34+ progenitor cells. The effect is stronger when the cells were cultured in RC. This is in accordance with the data for the synergizing effect of SCF, IL-3 and IFN-γ. The CFU-E were less affected by this cytokine possibly because of their higher degree of maturity making them less sensitive.

SEM is a necessary additional method for a detailed investigation of the erythroid cell morphology within the colonies. It allows the researcher to observe the erythroid cell-to-cell interactions in situ.

References

1. A l l e n, D. A., C. B r e e n, M. M. Y a q o o b, I. C. M a c d o u g a l l. Inhibition of CFU-E colony formation in uremic patients with inflammatory disease: role of IFN-gamma and TNF-alpha. – J. Investig. Med., **47**, 1999, No 5, 204-211.

2. Atlas of Human Hematopoietic Colonies. Vancouver, Canada, Stem Cell Technologies Inc, 1995.
3. Caux, C., I. Moreau, S. Saeland, J. Banchereau. Interferon-γ enhances factor-dependent myeloid proliferation of human CD 34+ hematopoietic progenitor cells. – Blood, **79**, 1992, No 10, 2628-2635.
4. Coutinho, L. H., N. G. Testa, T. M. Dexter. The myelosuppressive effect of recombinant interferon γ in short-term and long-term marrow cultures. – Br. J. Haematol., **83**,1986, 517-524.
5. Dai, C. H., S. B. Krantz, K. Kollar, J. O. Price. Stem cell factor can overcome inhibition of highly purified human burst-forming units-erythroid by interferon γ. – J. Cell. Phys., **165**, 1995, 323-332.
6. Flores-Guzman, P., M. Gutierez-Rodriguez, H. Mayani. *In vitro* proliferation, expansion and differentiation of a CD 34+ cell-enriched hematopoietic cell population from human umbilical cord blood in response to recombinant cytokines. – Arch. Med. Res., **33**, 2002, No 2, 107-114.
7. Fruehauf, S., K. Srbic, R. Seggewiss, J. Topaly, A. D. Ho. Functional characterization of podia formation in normal and malignant hematopoietic cells. – J. Leuk. Biol., **71**, 2002, 425-432.
8. Muta, K., S. Krantz, M. Bondurant, C. H. Dai. Stem cell factor retards differentiation of normal human erythroid progenitor cells while stimulating proliferation. – Blood, **86**, 1995, No 2, 572-580.
9. Shiohara, M., K. Koike, T. Nakahata. Synergism of interferon-γ and stem cell factor on the development of murine hematopoietic progenitors in serum-free culture. – Blood, **81**, 1993, No 6, 1435-1441.
10. Tsanev, R., I. Ivanov. immune Interferon – properties and clinical applications. CRC Press LLC, Boca Raton, 2002.
11. Wang, C.Q, K.B. Udupa, D.A. Lipschitz. Interferon-gamma exerts its negative regulatory effect primarily on the earliest stages of murine erythroid progenitor cell development. – J. Cell. Physiol., **162**, 1995, No 1, 134-138.