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The Impact of Human Recombinant Interferon-γ on the Capacity of the Human CD 34+ Hematopoietic Progenitor Cells to Differentiate *in vitro* into Mature Bone Marrow Macrophages

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Interferon-gamma (IFN-γ) stimulated the formation of granulocyte/macrophage- and pure macrophage colonies when added to the culturing medium. The stimulatory effect was better observed when the purified and enriched human CD34+ hematopoietic progenitor cells were cultured in Agarleucocyte conditioned medium (Agar-LCM).

Key words: CD34+ hematopoietic progenitor cells; IFN-γ; granulocyte/macrophage-(CFU-GM) and macrophage-(CFU-M) colonies.

Introduction

Hematopoiesis depends on continuous proliferation, expansion and differentiation of hematopoietic stem cells and their immediate progeny — hematopoietic progenitor cells. The two subsets of cells express CD 34 antigen — regulator of hematopoietic cell adhesion to stromal cells in the bone marrow [5]. CD34+ cell population comprise approximately 1% of the cells present in the bone marrow. Progenitor cells are also called colony forming cells (CFC) because of their ability to form colonies or clusters (bursts) during proliferation and differentiation when cultured in semi-solid medium [3]. In order to proliferate and differentiate into mature blood cells the progenitor cells require specific growth factors [10, 5]. It is a large group of proteins known as hematopoietic cytokines. According to Ogawa [12] the hematopoietic growth factors can be divided into 3 groups based on the stage of cell development which they affect: **late-acting, lineage-specific factors** — G-CSF, Epo, IL-5, IL-6; **intermediate-acting, lineage non-specific** — IL-3, IL-4, GM-CSF and **factors which affect the kinetics of cell cycle dormant primitive progenitors** — SCF, IL-6, IL-11, IL-12, G-CSF. Based on the effect of the cytokines - stimulatory or

inhibitory of the processes of cell proliferation and differentiation, the hematopoietic growth factors can be further divided into two groups: *stimulators* and *inhibitors* [12]. The most widely used cytokines known to stimulate hematopiesis *in vitro* are: stem cell factor (SCF), erythropoietin (Epo), different colony-stimulating factors (CSF). TNF-alpha, TGF-beta and IFN-γ act primarily as inhibitors.

IFN-γ is an inflammatory cytokine produced by activated T-lymphocytes, macrophages and *natural killer cells*. There are data [1, 2, 3, 13] showing its inhibitory effect on *in vitro* hematopoietic colony formation, and its ability to synergize with SCF. On the other hand, IFN-γ stimulates formation and maturation of granulocyte/macrophage- and macrophage colonies [4, 8, 9, 11]. It is suggested that the effects of this inflammatory cytokine depend on its dose, duration of time, the other factors present in the medium and the stage of maturity of the cells cultured [14].

Material and Methods

Cell cultures

Purified (density of 2.5×10^3/well) and enriched (density of 0.4×10^5/well) human CD34+ hematopoietic progenitor cells were cultured in semi-solid agar cultures. Two experimental systems were developed: IMDM was supplemented with SCF, IL-3 (called *recombinant cocktail - RC*), Epo or with Agar-stimulated leukocyte conditioned medium (*Agar-LCM*). IFN-gamma was added at 5000 U/ml once or at 200 U/ml 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 and observed by light microscopy after staining with May-Grünwald-Giemsa.

Results

In the *semi-solid agar cultures* can be observed:
— *CFU-E* — smallest and most rapidly maturing erythroid colonies, consisting of 1 or 2 clusters and containing maximum 100-200 erythroblasts.

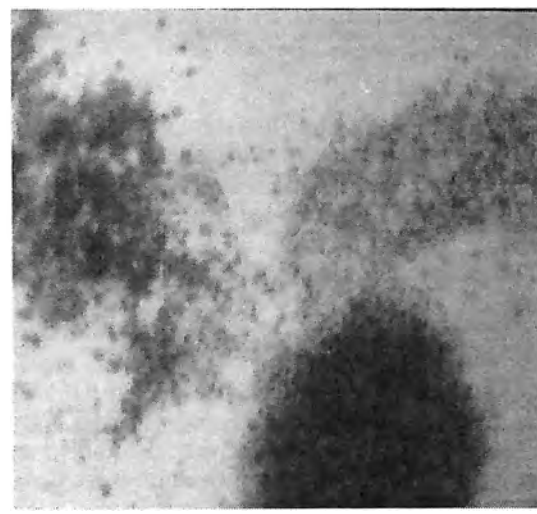

Fig. 1. IFN-γ induced three types of hematopoietic colonies: macrophage (M-); erythroid — (BFU-E); mixed granulocyte/macrophage — (GM-); May-Grünwald-Giemsa staining (× 160)

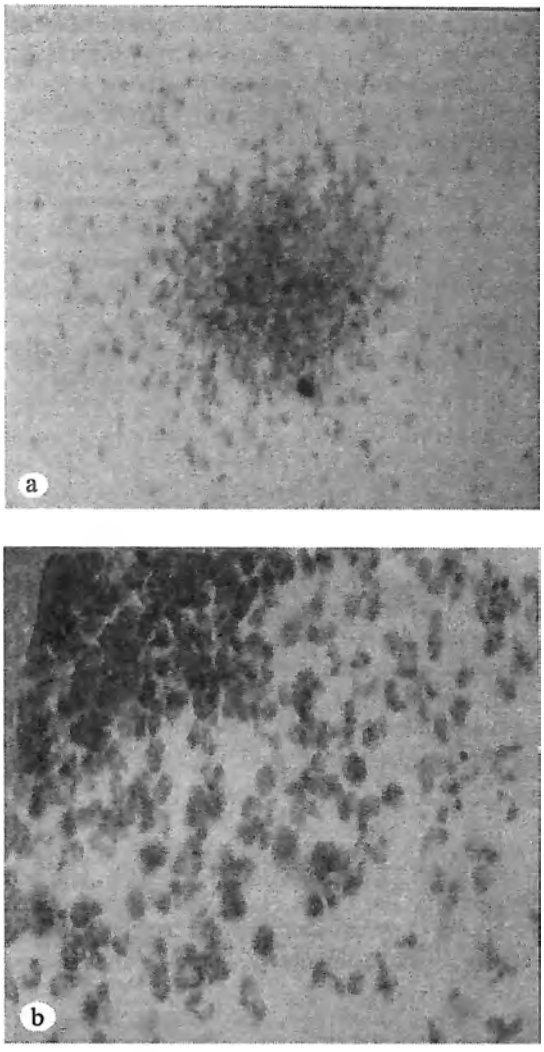

Fig. 2. **a)** Immature granulocyte/macrophage colony **(a)** and a part of the same colony containing granulocytes and macrophages in different stages of maturity **(b)**; Part of the same colony; granulocytes and macrophage can be seen; May-Grünwald-Giemsa staining (× 160; × 400)

— *BFU-E* — more primitive than CFU-E, give rise to larger, multi-clustered erythroid colonies which consist of 1 large or 3 small clusters (**Fig. 1**). *BFU-E* can be further divided into *mature* and *primitive BFU-E.*

— *CFU-GM* — with concentrated central core surrounded by a less dense halo of cells.

In all cases where *IFN-γ* was added a *stimulatory effect* only *on BFU-E and CFU-GM* formation was observed; no such effect was registered for the CFU-E colonies [6].

IFN-γ - added to cultured *in vitro* human CD34+ hematopoietic progenitor cells, *stimulated* (in a dose-dependent manner) *proliferation* and *differentiation* of *CFU-GM* and *CFU-M*. IFN-γ induced macrophage differentiation in the pure macrophage- (M-) and mixed — granulocyte/macrophage (GM-) colonies: a large number of mature, well-differentiated macrophages with vacuolated cytoplasm, more than one nucleus and dispersed (active) nuclear chromatin were observed (**Fig. 1c; 2a, b; 3a, b**).

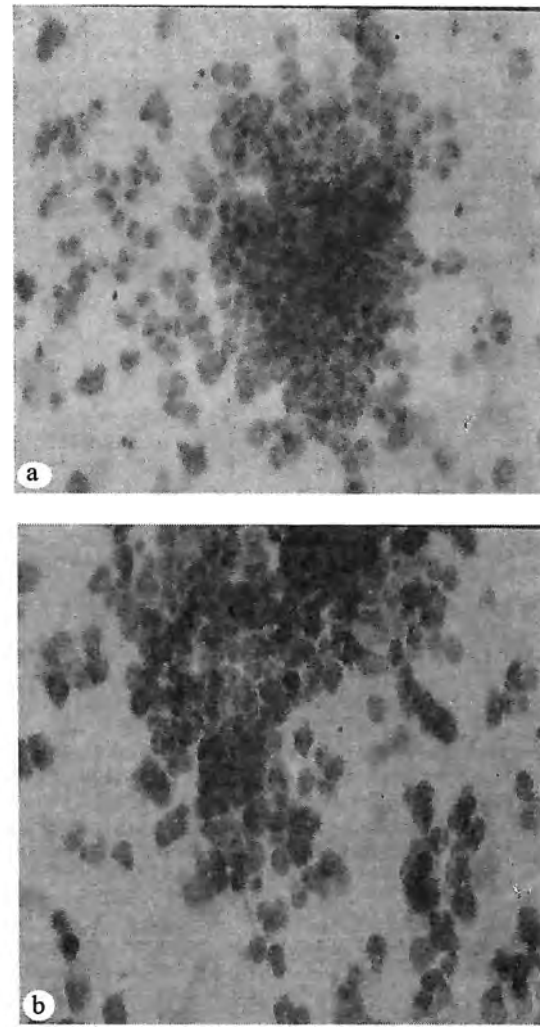

Fig. 3. Mature granulocyte/macrophage colony (a) and a part of the same colony (b) May-Grünwald-Giemsa staining (× 160, × 400)

Discussion

It was found that IFN-γ potentiates the proliferative effects of IL-3 and GM-CSF on CD34+ hematopoietic progenitor cells purified from cord blood and bone marrow [2]. Additionally, cell analysis showed that this effect of the cytokine is directly mediated on its target cells: day 14 colony assays showed that IFN-γ strongly potentiates both monocytic and granulocytic series, as it affects the erythroid lineage.

The results of the present study are in agreement with these of Caux et al. [2], Kawano et al. [7] and clearly show that this inflammatory cytokine appears to potentiate early myelopoiesis and the development of mature CFU-GM. The observed by us *in vitro* differences in the regulated by IFN-γ bone marrow macrophage development and maturation may contribute to the understanding of the macrophageal functional heterogeneity *in vivo* [8].

Conclusions

IFN-γ stimulated (in a dose-dependent manner) *proliferation* of *CFU-GM* and *CFU-M* when added to cultured *in vitro* human CD34+ hematopoietic progenitor cells. The better observed stimulatory effect of IFN-γ on cells cultured in *Agar-LCM* was likely due to the presence of *colony stimulating factors* (*CSFs*) in the conditioned medium used. The IFN-γ-induced *macrophage differentiation* (*GM-* and *M-colonies in different stages of maturity*) possibly contributes to the morphological and functional heterogeneity of these cells in the human bone marrow.

References

1. Broxmeyer, H. E. et al. Comparative analysis of the influences of human γ, α and β interferons on human multipotential (CFU-GEMM), erythroid (BFU), and granulocyte-macrophage (CFU-GM) progenitor cells. — J. Immunol., **131**, 1983, No 3, 1300-1305.
2. Caux, C. et al. Interferon-γ enhances factor-dependent myeloid proliferation of human CD 34+ hematopoietic progenitor cells. — Blood, **79**, 1992, No 10, 2628-2635.
3. 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, **63**, 1986, 517-524.
4. Delneste, Y. et al. Interferon-γ switches monocyte differentiation from dendritic cells to macrophages. — Blood, **101**, 2003, No 1, 143-150.
5. Flores-Guzman, P., M. Gutierrez-Rodriguez, H. Mayani. *In vitro* proliferation, expansion, and differentiation of a CD34+ cell-enriched hematopoietic cell population from human umbilical cord blood in response to recombinant cytokines. — Arch. Med. Res., **33**, 2002, No 2, 107-114.
6. Gluhcheva, Y. et al. Influence of interferon-gamma on human CD34+ hematopoietic progenitor cells. — Pteridines, **15**, 2004, No 2, 50-51.
7. Kawano, Y. et al. Synergistic effect of recombinant interferon-γ and interleukin-3 on the growth of immature human hematopoietic progenitors. — Blood, **77**, 1991, No 10, 2118-2121.
8. Lucas, D. M. et al. Analysis of the IFN-γ-signalling pathway in macrophages at different stages of development. — J. Immunol., **160**, 1998, 4337-4342.
9. McDowell, M. A. et al. Different utilization of IFN-γ responsive elements in two maturationally distinct MP cell lines. — J. Immunol., **155**, 1995, No 10, 4933-4938.
10. Metcalf, D. Hematopoietic regulators: redundancy or subtlety? — Blood, **82**, 1993, 3515-3523.
11. Nicolet, C. M., D. M. Paulnock. Promoter analysis of an interferon-inducible gene associated with MP activation. — J. Immunol., **152**, 1994, No 1, 153-163.
12. Ogawa, M. Differentiation and proliferation of hematopoietic stem cells. — Blood, **81**, 1993, 2844-2853.
13. 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.
14. Tsanev, R., I. Ivanov. Immune Interferon — properties and clinical applications. Boca Raton, CRC Press LLC, 2002.