Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 13
Sofia • 2008

IFN-γ Colony-Stimulating Activity and *In Vitro* Effects on Neopterin Synthesis and Tryptophan Degradation

Y. Gluhcheva, E. Zvetkova, G. Konwalinka, D. Fuchs**

Institute of Experimental Morphology and Anthropology with Museum, Bulgarian Academy of Sciences
** Medical University, Innsbruck, Austria*

IFN-γ stimulated *in vitro* hematopoietic (erythroid and myeloid) colony formation by cultured human CD34+ hematopoietic progenitor cells, as well as their neopterin production and tryptophan degradation. Elevated neopterin (NP) concentrations in the liquid layer of the semi-solid agar cultures corresponded to enhanced hematopoietic colony formation, decreased amounts of tryptophan (Try) and increased kynurenine concentrations. When the quantitative changes of both substances — neopterin and tryptophan, were compared, they showed linear statistical relation. Neopterin concentrations — compared to kynurenine/tryptophan ratio, gave exponential relation.

Key words: human CD34+ hematopoietic progenitor cells, semi-solid agar cultures, IFN-γ, neopterin production, tryptophan degradation.

Introduction

IFN-γ stimulates *in vitro* not only hematopoiesis but also the production and/or degradation of some biologically-active substances such as neopterin, kynurenine, tryptophan, etc. [1-3, 8]. Recent data [6, 7] suggested a correlation between tryptophan degradation and neopterin synthesis in cases of different neurodegenerative diseases. Positive correlation between NP and K/T is found in cases of anemia, HIV infection and some autoimmune diseases [4].

The aim of the study was to determine the *in vitro* colony-stimulating activity of IFN-γ on human CD34+ hematopoietic progenitor cells and the statistical relation between the concentrations of neopterin and tryptophan in the liquid layer of the semi-solid agar cultures.

Material and Methods

Human purified and enriched CD34+ hematopoietic progenitor cells (isolated from mobilized peripheral blood of myeloma patient) were cultured in semi-solid agar

cultures in IMDM — supplemented with SCF, IL-3, erythropoietin /Epo/ (called recombinant cocktail — RC) or with 20% Agar-stimulated leukocyte conditioned medium (Agar-LCM). IFN-γ was added to both experimental systems at a single dose — 5000 U/ml or at doses 200 and 400 U/ml, applied every second day. CD34+ hematopoietic cell cultures were incubated for 14 days at 37°C in humidified air of 5% CO_2. After incubation cell colonies and clusters were stained with May-Grünwald-Giemsa and observed by light microscope. The liquid overlayers of agar cultures were used for measuring neopterin and tryptophan concentrations. NP concentrations were determined by ELISA (ELItest®Neopterin Screening — BRAHMS Diagnostica, Berlin, Germany). *In vitro* tryptophan concentrations and kynurenine amounts respectively, were measured by HPLC [5]. Regression analysis was applied for determining the statistical relation between neopterin and trytophan concentrations in both experimental systems (hematopoietic cells cultured in RC and/or Agar-LCM).

Results

The addition of different doses IFN-γ to the semi-solid agar cultures stimulated hematopoietic (erythroid and myeloid) colony and cluster formation by purified and enriched human CD34+ progenitor cells growing in RC and Agar-LCM (Tables 1, 2).

T a b l e 1. Total number of hematopoietic colonies formed by purified CD34+ human progenitor cells

Culturing conditions	Controls	5000 U/ml IFN-γ - once	400 U/ml/ IFN-γ 2d	200 U/ml/ IFN-γ 2d
Agar-LCM	75	106	93	92.5
RC	89	100.5	122.75	118.5

T a b l e 2. Total number of hematopoietic colonies formed by enriched CD34+ human progenitor cells

Culturing conditions	Controls	5000 U/ml IFN-γ - once	400 U/ml/ IFN-γ 2d	200 U/ml/ IFN-γ 2d
Agar-LCM	60.2	85	100.75	99.75
RC	120	177	174.75	169.25

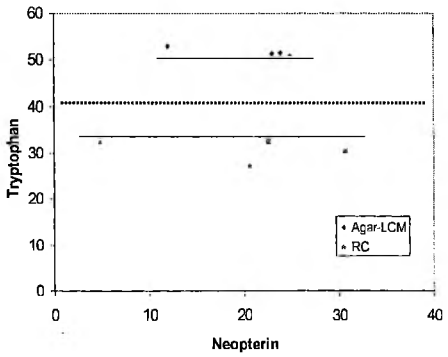

Fig. 1. Relation between neopterin production and tryptophan degradation by cultured *in vitro* purified CD34+ hematopoietic progenitor cells

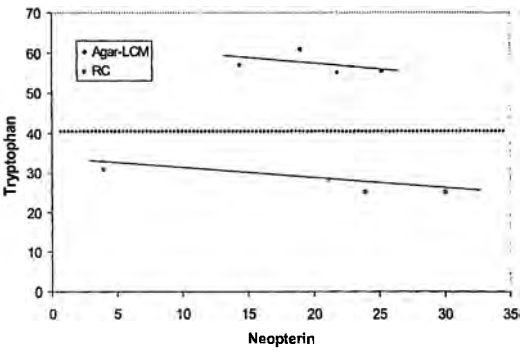

Fig. 2. Relation between neopterin production and tryptophan degradation by cultured *in vitro* enriched CD34+ hematopoietic progenitor cells

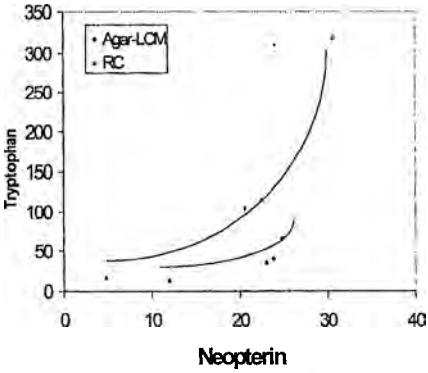

Fig. 3. Relation between neopterin production and kynurenine/tryptophan (K/T) ratio by cultured *in vitro* purified CD34+ hematopoietic progenitor cells

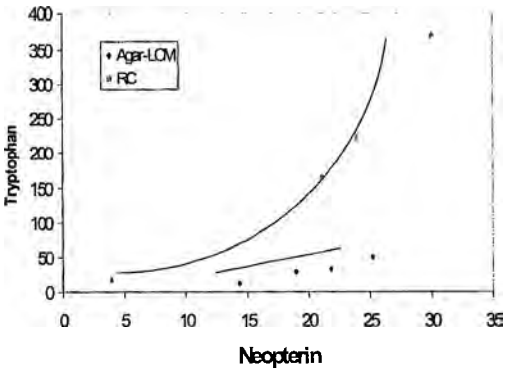

Fig. 4. Relation between neopterin production and kynurenine/tryptophan (K/T) ratio by cultured *in vitro* enriched CD34+ hematopoietic progenitor cells

When compared the concentrations of both biologically-active substances — NP and Try, a linear statistical relation was determined (Figs. 1, 2). The relation was exponential, when the logarithmic values of tryptophan/kynurenine (K/T) ratio and NP concentrations was compared (Fig. 3, 4). The enhanced colony formation under the *in vitro* influence of IFN-γ, corresponded to lower Try concentrations and elevated amounts of NP in the liquid layers of cultures.

Discussion

IFN-γ stimulated the *in vitro* formation of erythroid and myeloid colonies in both agar culture systems used — RC and Agar-LCM. The effects of the cytokine are better expressed when the CD34+ hematopoietic progenitor cells were cultured in RC where more erythroid colonies were obtained. The culture medium Agar-LCM enhanced myeloid (granulocyte/macrophage) cell proliferation, differentiation and colony formation. IFN-γ stimulated hematopoietic cell NP production and Try degradation in both culture systems. Our results are in agreement with those of W i r l e i t n e r et al. [7], showing in liquid cultures that increased NP concentrations correspond to decreased amounts of Try. The statistical correlation between the concentrations of both biologically-active substances — NP and Try is possibly due to simultaneous enzyme activation of GTP cyclohydrolase I (for neopterin production) and IDO (for tryptophan degradation) by IFN-γ, as is shown by T a y l o r et al. [3] for macrophages.

Conclusions

IFN-γ stimulates *in vitro* human CD34+ hematopoietic progenitor cell proliferation, differentiation and colony formation in semi-solid agar cultures. The cytokine activates the *in vitro* production of biologically-active substances — neopterin and kynurenine, used as biochemical markers for cellular activation. The quantitative relations determined between cellular NP synthesis and Try degradation may be used to predict the *in vitro* concentrations of either one of these substances.

References

1. Mellor, A. L., D. B. Keskin, T. Johnson, P. Chandler, D. H. Munn. Cells expressing indoleamine 2,3 dioxygenase inhibit T cell responses. — J. Immunol., **168**, 2002, No8, 3771-3776.
2. 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, No6, 1435-1441.
3. Taylor, M. W., G. Feng. Relationship between interferon-γ, indoleamine 2,3-dioxygenase, and tryptophan catabolism. — FASEB J., **5**, 1991, No11, 2516-2522.
4. Weiss, G., K. Schroecksnadel, V. Mattle, C. Winkler, G. Konwalinka, D. Fuchs. Possible role of cytokine-induced tryptophan degradation in anaemia of inflammation. — Eur. J. Haematol., **72**, 2004, No2, 130-134.
5. Widner, B., E. R. Werner, H. Schennach, H. Wachter, D. Fuchs. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. — Clin. Chem., **43**, 1997, No12, 2424-2426.
6. Widner, B., M. Ledochowski, D. Fuchs. Interferon-γ-induced tryptophan degradation: neuropsychiatric and immunological consequences. — Curr. Drug Metabol., **1**, 2000, No2, 193-204.
7. Wirleitner, B., G. Neurauter, K. Schroecksnadel, B. Frick, D. Fuchs. Interferon-γ-induced conversion of tryptophan: immunologic and neuropsychiatric aspects. — Curr. Med. Chem., **10**, 2003, No16, 1581-1591.
8. Wachter, H., D. Fuchs, A. Hausen, G. Reibnegger, E. R. Werner. Neopterin as a marker for activation of cellular immunity: immunologic basis and clinical application. — Adv. Clin Chem., **27**, 1989, 81-141.