Pteridines
Vol. 12, 2001, pp. 135 - 139

Neopterin Acting as a Bone Marrow Stem Cell Factor on Early Common Haematopoietic (Myeloid) and Stromal (Dendritic, CD34+) Cell Progenitors *in vitro*

Zvetkova E.,[1] Fuchs D.,[2] Katzarova E., Bakalska M.,[1] Svetoslavova M.,[1] Nikolov B.[1]

[1]Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Science, Sofia, Bulgaria
[2]Institute of Medical Chemistry and Biochemistry, Leopold Franzens University, Innsbruck, Austria

Abstract

The *in vitro* response of early haematopoietic progenitors or stem cells, common for myeloid (granulocyte, eosinophil, megacaryocyte) and marrow stromal (including dendritic CD34+) cells series, to neopterin, exogenously added to the liquid- and semi-liquid (agar-) mouse bone marrow cultures, at doses 12.5 - 25 μg/ml culture medium, has been studied. The results obtained show a significant stimulation of common myeloid and stromal dendritic cell (CD34+) progenitors' proliferation and/or differentiation as early as 24h after the *in vitro* treatment of marrow cultures with neopterin. On day 4 of cultivation the granulocyte/macrophageal proliferation and differentiation has been attenuated giving place to the marrow stromal dendritic cell (probably CD34+) differentiation. The engagement of the nuclear proliferative factor NF-κ B may play a role in the light of recent data that neopterin could activate this transcriptionally active nuclear factor. The significance of clonal selective stimulation of healthy (but not of leukaemic) dendritic CD34+ cells in some pathological cases as acute myeloid leukaemia has been also discussed because neopterin could be a more efficient marrow stem cell factor than cytokine cocktails with granulocyte/macrophage-colony stimulating factor. The *ex vivo* or clinical application of neopterin, alone and/or in specific combinations with other cytokines, in the induction of marrow myeloid and stromal cell proliferation and differentiation towards the dendritic (CD34+) cells merits further investigations..

Key words: mouse, bone marrow, neopterin, marrow stem cell factor, haematopoietic progenitors, stromal dendritic cell progenitors, DC (CD 34+), nuclear factor-κ B

Introduction

Some marrow haematopoietic and stromal cells as these from granulocyte/macrophageal (G/M), megacaryocytic (Mg) and stromal dendritic cell (SDC) series could have a subset of common early marrow progenitors (e.g. CD34+), giving rise to blood and stromal cell growth and differentiation (1-3). Several cytokines as colony stimulating factors, macrophage colony stimulating factor M-CSF and granulocyte/macrophage colony stimulating factor GM-CSF, and interleukins IL-3 and IL-4 or cytokine cocktails contribute to marrow haematopoietic and/or stromal cell progenitors' growth and differentiation into macrophages, granulocytes/macrophages and stromal dendritic cells in normal and pathological (e.g., acute myeloid leukaemia) cases (3-7). On the other hand, there are data in the literature (8) that not only monocytes/macrophages (Mo/Ma), but also dendritic cells (DCs) in humans are the source of increased neopterin production, during states of immune activation as well as under influence of stimuli that lead to maturation of these cells. A functional role of neopterin for the proliferation and differentiation of bone marrow common haematopoietic and stromal progenitors is not yet clear and remains to be elucidated having also in mind our previous results on the *in vitro* haematopoietic and stromal dendritic cell stimulating effects of some ranopterins - pterin-6-carboxylic acid and neopterin (9-12).

The aim of the present work was to examine the *in vitro* response of very early and probably common haematopoietic and stromal cell (including DCs) marrow progenitors (CD34+) to neopterin exogenously

Correspondence to: Dr.Elissaveta Zvetkova, Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Sciences, Acad.G.Bonchev Str., Bl.25, 1113-Sofia, Bulgaria,
Fax: +359 2 719 007, E-mail: ezvetkova@hotmail.com

added to liquid and semi-liquid (agar-) mouse bone marrow cultures.

Materials and Methods

Solutions and Media, IMDM and fetal calf serum, were obtained from GIBCO. Bone marrow-derived cells (BMCs) preparation and cultivation *in vitro*: BMCs were prepared and cultured in conditions of liquid and semi-liquid (agar-) cultures by a modification of previously reported methods (13). Briefly, a single cell suspension of femural bone marrow cells (10.6 nucleated cells/ml culture medium) was cultured in starting medium as controls for 24, 48 and 72h. The *in vitro* response of bone marrow haematopoietic and stromal cell progenitors (precursors) to neopterin (Schircks Laboratories, Jona, Switzerland), exogenously added to the liquid and agar mouse bone marrow cultures (at doses 12.5-25 μg/ml medium) has been also examined after 24, 48 and 72h of cultivation. The non-adherent cells from controls and neopterin-treated liquid cultures were collected, cytocentrifuged and the cytospin preparations obtained have been stained (14) for DNP, RNP and some basic cytoplasmic and nuclear proteins. The same staining method, after modification, has been applied on plastics to visualise cell colonies and clusters of adherent haematopoietic and stromal marrow cells. The cytospin preparations, prepared from the liquid parts of the cultures, as well as cells stained on plastic dishes (Nunc) were examined with light microscopy. The agar cultures were fixed and stained on day 7 of cultivation according to Zvetkova and Jelineck (13) for the *in situ* staining of cell colonies and clusters in bone marrow semi-solid cultures.

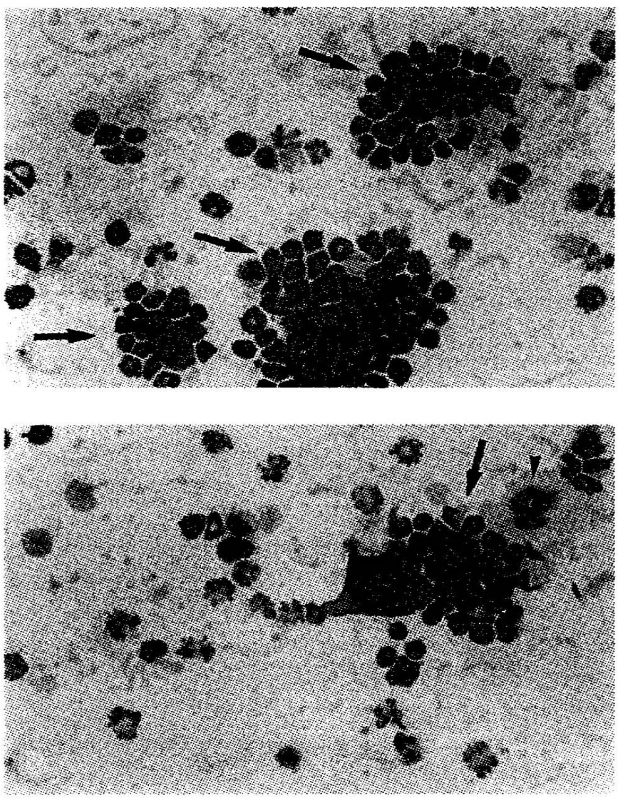

Figure 1. Cytospin preparations from the liquid mouse bone marrow culture, treated exogenously (24 h) with neopterin. One could see granulocyte/macrophageal colonies (arrows) with a single megacaryocyte (Mg-, arrow head) in one of them. Staining by the method of Zvetkova and Zvetkov; x 600 (14)

Results

The results obtained show a stimulation of myeloid (granulocyte, eosinophil, monocyte/macrophage, and megacaryocyte) cell proliferation and differentiation as early as 24h after the *in vitro* treatment with exogenously added neopterin to the liquid mouse bone marrow cultures (Fig.1). At this time the marrow stromal cells have not yet appeared and/or differentiated in liquid cultures as well as on the bottom of plastics: only rare stromal cells could be visible in some very early granulocytic and granulocyte-macrophageal colonies and clusters of adherent cells.

At day 4 of the *in vitro* marrow cultivation the macrophageal- and granulocytopoiesis (granulocyte/macrophage cluster and colony-formation) was attenuated giving place to intensive marrow stromal cell (including dendritic cells) proliferation and differentiation (Figs. 1,2). At this time one could see also

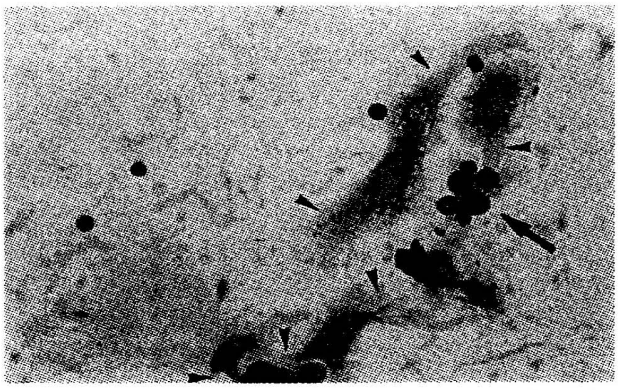

Figure 2. Bone marrow stromal cells stained in the bottom of the plastics (Petri dishes) in the long term bone marrow cultures (48 h), treated *in vitro* by neopterin. Stromal macrophages with enhanced adhesiveness, forming abundant cytoplasmic protrusions and long dendrite-like uropodes (arrow heads) are localized very near to the clusters of lymphocyte-like undifferentiated cells (early progenitors/stem cells; see - arrow). The same staining method with modificationx Zvetkova and Zvetkov; x 600 (14)

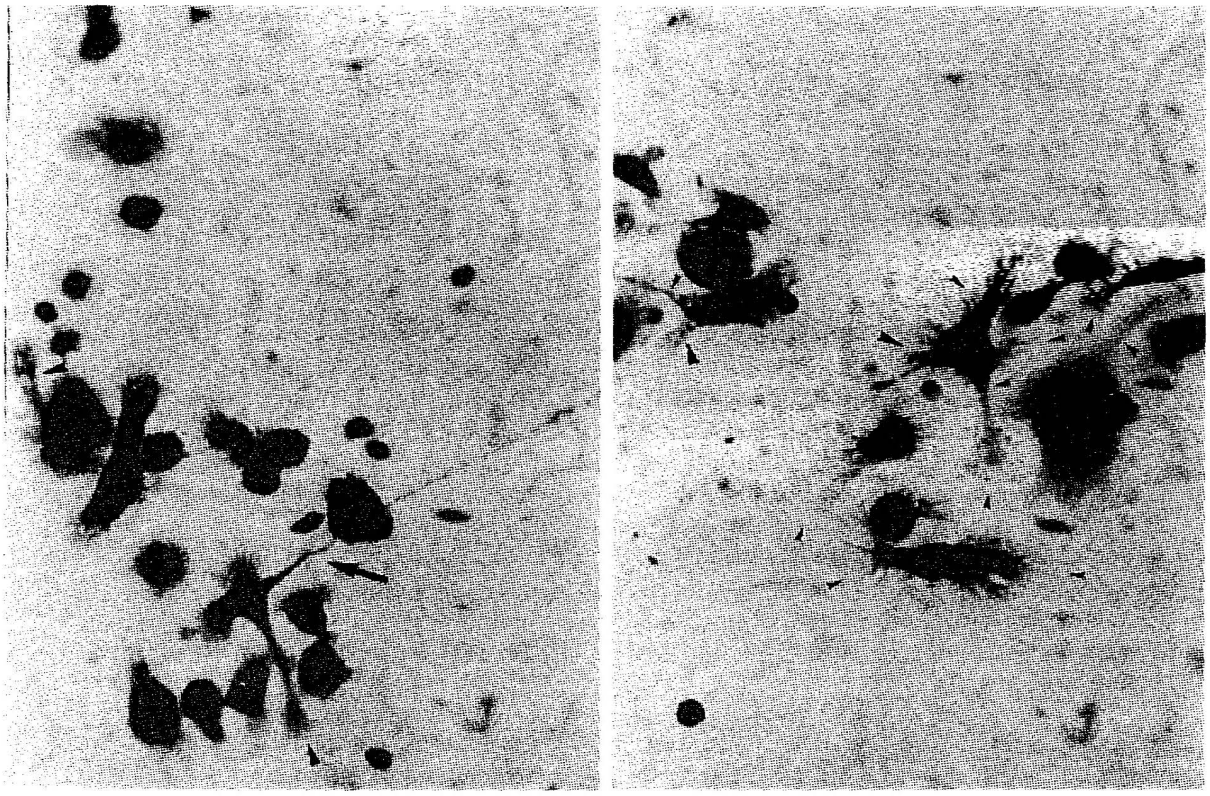

Figure 3. Granulocyte/macrophageal cluster with dendritic cells forming very long cytoplasmic dendrite-like protrusions (arrow) in mouse bone marrow agar culture (at day 7 of cultivation) - exogenously treated by neopterin. Staining by the method of Zvetkova and Jelinek; x 1250, Immersion (13)

clusters of undifferentiated lymphocyte-like cells, probably very early progenitors (stem cells), localized very near to the stromal DCs (Fig. 1, arrow).

At day 7 of mouse bone marrow cultivation in agar, the macrophageal-granulocytic colonies and cell clusters were obtained with single DCs in them (Fig. 3). The "healthy" mouse DCs appearing in our agar cultures only in cases of treatment with exogenous neopterin, could be morphologically compared with "pathological" ones, described previously (15) in the marrow agar cultures of patients with acute myeloid leukaemias.

Discussion

Neopterin is a metabolite of guanosine triphosphate and large amounts of it are produced at the expense of biopterin derivatives by monocytes/macrophages in response to stimulation with interferon-γ (8). Neopterin is a useful marker of immunological activation (5), but its biological haematopoietic activities are still unclear. Recently we found that ranopterins, Pterin-6-carboxylic acid and neopterin, stimulate granulocyte/macrophage colony formation *in vitro* in conditions of mouse bone marrow agar cultures, acting as MG-CSF and marrow stromal cell factor (MSCF), respectively (9-12).

In this work the effects of the synthetic neopterin on the haematopoietic stem cell proliferation and differentiation *in vitro* has been examined using the same models for mouse bone marrow cultivation and in situ staining. The results suggest that exogenously added neopterin stimulates simultaneously haemopoietic (myeloid) and stromal cell proliferation and differentiation, activating in a great degree stromal dendritic cell production probably from a common early CD34+ marrow progenitors/stem cell.

The data obtained 24h - 4 days after the addition of various concentrations of neopterin in marrow liquid cultures, as well as after 7 days cultivation in soft agar, show that neopterin acts as marrow stem cell factor and directly affects the proliferation of early common haematopoietic and stromal dendritic cell progenitors (probably CD34+). Such enhancing haematopoietic and stromal marrow cell activity of neopterin could be of importance in normal and pathological cases because bone marrow stromal cells have been shown to be essential in stimulation of haematopoiesis through production of humoral growth factors, cell-to-cell interactions or both. Our study shows that cell-to-

cell interactions between haematopoietic, stem cells including, and stromal dendritic cells (Fig. 2, arrow) could be obtained in very early haematopoietic colonies and clusters. The importance of these direct cell-to-cell interactions between haematopoietic and stromal cells during the processes of haematopoietic cell proliferation and differentiation *in vitro* could be elucidated more in the future in the light of recent data (4, 7, 15) about the importance of the so-called "healthy" CD34-positive clonal cells as well as the clonal reduction by cytokine cocktails of "pathological" CD34+ in cases with myeloid leukaemia.

The *in vitro* stimulatory effects of neopterin influencing proliferation and differentiation/maturation of early common bone marrow progenitors are probably displayed through the nuclear transcription factor NF-κ B (12). Activation of NF-κ B by neopterin and 7,8-dihydroneopterin has been demonstrated earlier in cells from rats and humans (18, 19). The engagement of this proliferative factor in the stimulation of CD34+ marrow stromal dendritic cells remains to be elucidated in further investigations.

Acknowledgments

This work is supported by Austrian Ministry of Education and Technology, under Contract with Austrian Science and Research Liaison, OSI, Office Sofia, August, 2000.

References

1 Banchereau, J., R. Steinman. Dendritic cells and the control of immunity. Nature 1988; 392: 245 - 252

2 Waller, E., J. Olweus, F. Lund-Johansen, S. Huang, M. Nguyen, G.R. Guo, L. Terstappen. The "Common Stem Cell" hypothesis reevaluated: Human fetal bone marrow contains separate populations of hematopoietic and stromal progenitors. Blood 1995; 85: 2422 - 2435

3 Young, J. W., P. Szabolcs, M. Moore. Identification of dendritic cell colony-forming units among normal CD34 bone marrow progenitors that are expanded by c-kit-ligand and yield pure dendritic cell colonies in the presence of granulocyte/macrophage colony-stimulating factor and tumor necrosis factor alfa. J. Exp. Med. 1995; 182: 1111 - 1120

4 Braun, S., H. Gerhartz, H. Schmetzer. Cytokines can reduce clonal, CD34-positive cells in acute myeloid leukemia in vitro. Ann. Hematol. 2000; 79: 363 - 373

5C aux, C. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF + TNF alfa: II. Functional analysis. Blood 1997; 90: 1458 - 1470

6 Caux, C., C. Dezutter-Dambuyant, D. Schmitt, J. Banchereau. GM-CSF and TNF-alfa cooperate in the generation of dendritic Langerhans cells. Nature 1992; 360: 258 - 261

7 Oehler, L., A. Berer, M. Kollars, F. Keil, M. Konig, M. Waclavicek, O. Haas, W. Knapp, K. Lechner, K. Geissler. Culture requirements for induction of dendritic cell differentiation in acute myeloid leukemia. Ann. Hematol. 2000; 79: 355 - 362

8 Romani, N., B. Widner, D. Reider, E. Ebner, B. Wirleitner, D. Fuchs. Are dendritic cells a relevant source for neopterin in humans? Pteridines 1999; 10: 61 - 62

9 Zvetkova, E., E. Janeva, E. Nikolova, G. Milchev, A. Dikov, I. Tsenov, N. Bojilova, A. I. Hadjioloff. Hemopoietic colony-stimulating activity of ranopterines in murine bone marrow agar cultures. Compt. Rend. Acad. Bulg. Sci. 1991; 44: 91 - 94

10 Zvetkova, E., E. Janeva, A. Dikov, A. I. Hadjioloff. Ranopterine with MG-CSF-like activity in murine bone marrow agar cultures. Cell Prolif. 1992; 25: 498

11 Zvetkova, E., I. Tsenov, E. Katzarova, M. Bratanov, P. Angelova, I. Chowdhury, B. Nikolov, A. Dikov. In vitro proliferation of mouse bone marrow lymphocytes and stromal macrophages under the biological action of ranopterin (neopterin). Compt. Rend. Acad. Bulg. Sci. 1995; 48: 81 - 84

12 Zvetkova, E. Ranopterins - Amphibia skin pteridines displaying hematopoietic, immunomodulatory, and macrophageal proliferative biological activities. Pteridines 1999; 10: 178 - 189

13 Zvetkova, E., J. Jelinek. Methylene blue-fast green staining of hemopoietic colonies in agar cultures. Gegenbaurs Morphol. Jahrb. 1989; 135: 779 - 793

14 Zvetkova, E., I. Zvetkov. A cytological method for the simultaneous staining of nucleoproteins and some cationic proteins. Acta Histochem. 1976; 57: 1 - 13

15 Zvetkova, E., J. Jelinek. Myeloid leukaemia predidictive, diagnostic and prognostic models in vitro based on the presence of undifferentiated granulocytes and atypical blast-like macrophages in bone marrow agar cultures. Compt. Rend. Acad. Bulg. Sci. 1997; 50: 105 - 108

16 Wachter, H., D. Fuchs, A. Hausen, G. Reibnegger, G. Weiss, E. Werner, G. Werner-Felmayer. Neopterin : Biochemistry, Methods, Clinical Application. Berlin-New York, Walter de Gruyter, 1992, pp. 1 - 294

17 Fuchs, D., A. Hausen, G. Reibneger, E. Werner, M. Dierich, H. Wachter. Neopterin as marker for activated cell-mediated immunity: application in HIV infection. Immunol. Today 1988; 9: 150 - 155

18 Hoffmann, G., W. Schobersberger, S. Frede, L. Pelzer, J. Fandrey, H. Wachter, D. Fuchs, J. Grote. Neopterin activates transcription factor nuclear factor-kB in vascular smooth muscle cells. FEBS Lett. 1996; 391: 181-184

19 Baier-Bitterlich, G., D. Fuchs, R. Zangerle, P. A. Baeuerle, E. R. Werner, F. Fresser, F. Überall, G. Baier, H. Wachter. trans-Activation of the HIV type 1 promoter by 7,8-dihydroneopterin in vitro. AIDS Res Human Retrovir 1997; 13: 173-178 Biol. Chem. 1974; 248: 4100-4103.