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Exogenously Added in Vitro Neopterin is the Bone Marrow Stem Cell Factor (BMSCF) Acting on the Early Common Hematopoietic (Myeloid) and Stromal (Dendritic — CD34+) Cell Progenitors

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The in vitro response of early hematopoietic progenitors or stem cells (SCs) — common for myeloid (GM-, Eos-, Mg-) and marrow stromal cell (including dendritic cell /DC - CD34+/) series, to neopterin (NeoPt), 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 the common — myeloid and stromal DC (CD34+) progenitors' proliferation and/or differentiation as early as the 24th h. of the in vitro experimental treatment of marrow cultures with NeoPt. On the day 4th of cultivation the GM-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-kappa B, has been discussed, in the light of recent data that NeoPt could activate this transcriptionally active nuclear factor. The significance of clonal selective stimulation of healthy (but not of leukaemic) dendritic — CD34-positive cells in some pathological cases as acute myeloid leukaemia (AML) has been also discussed because NeoPt could be more efficient as BMSCF than cytokine cocktails with GM-CSF. The ex vivo or clinical application of NeoPt — alone and/or in specific combinations with other cytokines, in the induction of marrow myeloid and stromal cell proliferation and differentiation — towards the DCs (CD34-positive cells), merits further investigations.

Key words: mouse bone marrow; neopterin (NeoPt); bone marrow stem cell factor (BMSCF) influencing early common hematopoietic and stromal dendritic cell (DC) progenitors; DC (CD 34+) clonal selective stimulation in normal and pathological (AML) cases; transcriptionally active nuclear proliferative factor — NF-kappa B

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Introduction

It is well known fact [1, 9, 10] that some marrow hematopoietic and stromal cells as these from granulocyte/macrophageal (G/M), megacaryocytic (Mg) and stromal dendritic cell (SDC) series could have subset of common early marrow progenitors (e.g. CD34+), giving rise to blood and stromal cell growth and differentiation. Several cytokines as M-CSF, GM-CSF, IL-3 and IL-4 or cytokine cocktails contribute to marrow hematopoietic and/or stromal cell progenitors' growth and differentiation: into macrophages, granulocytes/macrophages and stromal dendritic cells - in normal and pathological (AML) cases [2, 3, 4, 6, 10]. On the other hands, there are data in the literature [7] that not only monocytes/macrophages (Mo/M), but also dendritic cells (DCs) in humans are exactly the source of increased NeoPt production, during states of immune activation as well as under influence of stimuli that lead to maturation of these cells. A functional role of NeoPt for the proliferation and differentiation of bone marrow common hematopoietic and stromal progenitors is not yet clear and remains to be elucidated having also in mind our previous results on the in vitro hematopoietic and stromal dendritic cell stimulating effects of some ranopterins — Pt-6-COOH and neopterin [14 - 17].

The aim of the present work was to examine the in vitro response of very early and probably common — hematopoietic and stromal cell (DC including) marrow progenitors (CD34+) to NeoPt, exogenously added to the liquid and semi-liquid (agar-) mouse bone marrow cultures.

Material and Methods

Solutions and Media — IMDM and FCS ware 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 ours previously reported methods [12]. 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 72 h. The in vitro response of bone marrow hematopoietic and stromal cell progenitors (precursors) to neopterin (Schirks 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 72 h cultivation. The nonadherent cells from control and neopterin-treated liquid cultures were collected, cytocentrifuged and the cytospin preparations obtained have been stained by the method of Zvetkova and Zvetkov [11] 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 hematopoietic 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 light microscopically.The agar cultrures were fixed and stained on the day 7th of cultivation, after the method of Zvetkova and Jelineck [12] for the in situ staining of cell colonies and clusters in bone marrow semi-solid cultures.

Results

The results obtained show a stimulation of myeloid (granulocyte- /Gr-/, eosinophil-/Eo-/, monocyte/macrophage (Mo/Ma-) and megacaryocyte /Mg-/) cell proliferation and differentiation as early as at the 24th h of the in vitro experimental treatment with exogenously added NeoPt to the mouse bone marrow cultures (Fig.1). At this time the

Fig. 1. Cytospin preparation from the liquid mouse bone marrow culture, treated exogenously (24 h) with NeoPt. One could see GM-colonies (arrows) with a single megacaryocyte (Mg-, arrowheads) in one of them. Staining by the method of Zvetkova and Zvetkov; × 600

Fig. 2, 3. 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 NeoPt. Stromal macrophages with enhanced adhesiveness, forming abundant cytoplasmic protrusions and long dendrite-like uropodes are localized very near to the clusters of lymphocyte-like undifferentiated cells (early progenitors / stem cells; see Fig. 2 — arrow). The same staining method with modification, × 1250-immersion

marrow stromal cells were not yet appeared and/or differentiated in liquid cultures as well as on the bottom of the plastics: only rare stromal cells could be visible in some very early granulocytic (G-) and granulocyte-macrophageal (GM-) colonies and clusters of adherent cells.

At the day 2nd — 4th of the in vitro marrow cultivation the macrophageal- and gra-nulocytopoiesis (GM- cluster and colony-formation) was attenuated giving place to the intensive marrow stromal cell (including dendritic cells — SDCs) proliferation and differentiation (Fig. 2, 3). At this time one could see also clusters of undifferentiated lymphocyte-like cells — probably very early progenitors (stem cells), localized very near to the stromal DCs (Fig. 2 — arrow).

At the day 7th of mouse bone marrow cultivation in agar, the macrophageal-granulocytic (MG-) colonies and cell clusters were obtained with single DCs in them (Fig. 4). The "healthy" mouse DCs appearing in our agar cultures — only in cases of treatment with exogenous NeoPt, could be morphologically compared with "pathological" ones, described previously by us [13] in the marrow agar cultures of patients with acute myeloid leukaemias (AML).

Discussion

Neopterin is a metabolite of guanosine triphosphate in the synthetic pathway of biopterin and large amounts of it are produced by monocyte/macrophages (Mo/M) in response to stimulation with INF-gamma [8]. It is well known fact that the produced by monocytes pteridine NeoPt could be a useful marker of immunological activation [5], but its biological hematopoietic activities are still unclear. Recently, we found that ranopterins — Pteridine-6-carboxilic acid (Pt-6-COOH) and NeoPt stimulate GM-colony formation in vitro — in conditions of mouse bone marrow agar cultures, acting as MG-CSF and bone marrow stromal cell factor (BMSCF), respectively [14 - 17].

Fig. 4. GM-cluster with DCs, forming very long cytoplasmic dendrite-like protrusions in mouse bone marrow agar culture (at the day 7th of cultivation) — exogenously treated by NeoPt. Staining by the method of Zvetkova and Jelinek; ×450

In this work the effects of the synthetic NeoPt on the hematopoietic stem cell proliferation and differentiation in vitro have been examined using the same our own models for mouse bone marrow cultivation and in situ staining. From the results obtained it could be suggested that exogenously in vitro NeoPt treatment stimulates simultaneously hemopoietic (myeloid) and stromal cell proliferation and differentiation, activating in a great degree stromal dendritic cell (SDC-) production — probably from a common early marrow progenitors (stem cells — CD34+).

The data obtained 24 h — 4th days after the addition of various concentrations of NeoPt in marrow liquid cultures, as well as after 7 days cultivation in a soft

agar, show that NeoPt, acting as bone marrow stem cell factor (BMSCF) directly affect the proliferation of early common - hematopoietic and stromal dendritic cell progenitors (probably CD34+). Such enhancing hematopoietic and stromal marrow cell activity of NeoPt could be of importance in normal and pathological cases because the bone marrow stromal cells have been shown to be essential in stimulation of hematopoiesis through production of humoral growth factors, cell-to-cell interactions or both. Our study showed that cell-to-cell interactions between hematopoietic, stem cells including, and stromal dendritic cells (See Fig. 2 — arrow) could be obtained in very early hematopoietic colonies and clusters. The importance of these direct cell-to-cell interactions between hematopoietic and stromal cells during the processes of hematopoietic cell proliferation and differentiation in vitro could be more elucidated in the future in the light of recent data [2, 6, 13] 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 stimulative effects of NeoPt influencing proliferation and differentiation/maturation of early common bone marrow progenitors are probably displayed through the nuclear transcription factor — NF-kappa B [17]. The engagement of this proliferative factor in the stimulation of marrow stromal dendritic cells (DCs — CD34+) remains to be ellucidated in further investigations.

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