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Hematopoietic Growth Factors

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This mini-review describes the affects (stimulatory and inhibitory) of some of the most common hematopoietic factors in the processes of proliferation and differentiation of the bone marrow progenitor cells (CD34+).

Key words: hematopoietic growth factors, bone marrow hematopoiesis, myelopoiesis, erythropoiesis, bone marrow progenitor cells (CD34+).

Haemopoiesis is a useful model system for studying differentiation and regulation of precursor cell populations - intermediate between the multipotent stem cell and terminally differentiated end cells [11]. Bone marrow blood cell production – hematopoiesis, depends on the continuous proliferation, expansion, and differentiation of a small subset of primitive cells that includes both hematopoietic stem cells (HSCs) and their immediate progeny (hematopoietic progenitor cells – HPCs).

1. Hematopoietic factors influencing white blood cell's formation

It is determined that survival and proliferation of cultured *in vitro* marrow progenitor cells depend on the presence of different hematopoietic cell factors.

The cytokines are the main growth factors that affect hematopoiesis by stimulating or inhibiting it. The interleukins and the colony-stimulating factors (CSFs) are part of the group of components that stimulate the process of formation of blood cells. On the contrary, factors such as tumor-necrosis factor-α (TNF-α), interferon-γ (IFN-γ), macrophage inflammatory protein-1α (MIP-1α), lactoferin, transforming growth factor-β (TGF-β) etc., inhibit hematopoiesis. It is considered that the group of cytokines probably has also a very important role in bone marrow morphogenesis. The results from different investigations indicate that the distribution of the hematopoietic cells depends on the presence of regulatory factors and their ability to diffuse in the bone marrow [23].

The **granulocyte-macrophage colony stimulating factor (GM-CSF)** influences the process of granulocytopoiesis. It is a glycoprotein with a molecular weight of 22 kDa. Its gene is localized in the long arm of the fifth chromosome. This protein is pro-

duced by activated T-lymphocytes, macrophages and mesenchymal cells. GM-CSF stimulates *in vitro* the formation of cell colonies of neutrophils, macrophages as well as mixed colonies (neutrophil-macrophageal and eosinophil). Under certain conditions it can also affect the formation of colonies if megakaryocytes and erythrocytes and can protect the differentiation of macrophages, neutrophilic and eosinophilic granulocytes.

The **granulocyte-colony stimulating factor (G-CSF)** is a 19-kDa glucoprotein. Its gene is localized on the 17th chromosome. This factor is produced by monocytes, fibroblasts, endothelial cells and it stimulates the in vitro growth of colonies of neutrophils. During hematopoiesis the G-CSF's receptor is specifically expressed on the surface of granulocytic precursors and on the mature granulocytes. This protein can induce the pluripotent stem cells to enter into the cell division cycle. G-CSF is applied in the treatment of different haematological diseases as a stimulator of white blood cells' proliferation [23].

The **macrophage-colony stimulating factor (M-CSF)** is a specific cell growth factor influencing the differentiation of marrow monocytes and macrophages. It is produced by the endothelial cells, fibroblasts and monocytes and has been isolated from murine L-cells and human urine as a homodimer with a molecular weight of 45 kDa [23]. Its gene is localized in the long arm of the chromosome 1. It has been established that this cell factor stimulates the *in vitro* formation of macrophageal colonies and that it influences the process of maturation of the mononuclear phagocytes by stimulating the cellular synthesis of RNA and proteins. On the other hand, M-CSF stimulates the production of monokines, including interferon, TNF and IL-1; it also enhances the cell-mediated cytotoxicity and synergises the effects of Il-1 and IL-3.

The **stem cell factor (SCF, c-KIT Receptor, Steel factor)** is a protein – similar to the lymphopoietic growth factor-1. It has a thyrosine-kinase activity and synergises with the stimulatory effects of other cytokines on the bone marrow stem cells. The stem cell factor is important for the survival of the non-dividing hematopoietic stem cells and also stimulates the proliferative and functional activity of the mast cells. This factor is a key regulator of the hematopoiesis. TNF-α can inhibit directly the stimulated with SCF proliferation of CD34+ hematopoietic progenitor cells.

Interleukin-1 (IL-1) is produced mainly by activated (stimulated) macrophages and monocytes, as well as by fibroblasts, endothelial and other cells types. IL-1 can increase the influence of different growth factors affecting the hematopoietic progenitor cells (alone or with the release of IL-6). On the other hand it can stimulate the synthesis of hematopoietic growth factors such as GM-CSF and G-SCF [23]. **Interleukin 2 (IL-2)** is the first defined T-cell growth factor. IL-2 is produced by activated CD4+ T- or CD8+ T- and NK-cells. It stimulates synthesis of other cytokines such as IFN-γ; but inhibits formation of GM-colonies and bone marrow erythropoiesis [7, 24]. **Interleukin 3 (IL-3)** is known as multi-CSF. It is a glycoprotein with a molecular weight of 15-25 kDa. Il-3 is produced by activated T-lymphocytes. It stimulates the proliferation of pluripotent myeloid cells. The gene encoding IL-3 is localized on the long arm of chromosome 5. Il-3 stimulates the early precursors of G- and M-, although its affect differs from that of the GM-CSF. It is also well known fact that IL-3 stimulates generation of macrophage, neutrophil, eosinophil, mast cell, erythroid and megakaryocytic colonies in human bone marrow [23]. **Interleukin 4 (IL-4)** is significant for the immune response cell growth factor, affecting B-, T-cells and macrophages. Besides the ability of IL-4 to stimulate proliferation of thymocytes and T-lymphocytes, mast cells, hematopoietic cells etc., it can inhibit the expression of IL-1 and TNF-α from peripheral blood monocytes. IL-4 regulates the NP production by adherent mononuclear cells [22, 30]. **Interleukin 5 (IL-5)** stimulates B-cells' blast transformation to plasmocytes which produce immunoglobulins. The action of this cytokine is identical with the this of the eosinophil differ-

entiation factor (EoDF), stimulating the processes of proliferation, differentiation and functions of the eosinophilic granulocytes [23]. **Interleukin 6 (IL-6)** is a 26-kDa glycoprotein with different functions. Il-6 (like IL-1 and G-CSF), stimulates marrow progenitor cells to enter the G1-phase of the cell cycle. It is believed that IL-6 can be applied clinically (with another CSF) for maximal stimulation of hematopoiesis in humans. **Interleukin 7 (IL-7)** is known as lymphopoetin-6. It triggers the proliferation of B-lymphocytes in the bone marrow, affects T-lymphocytes in the peripheral blood and activated murine thymocytes. **Interleukin 8 (IL-8)** or neutrophil-activating peptide causes granulocytosis and in some cases - a massive mobilization of hematopoietic stem cells. **Interleukin 9 (IL-9)** affects the formation of erythroid burst-forming unit (BFU-E) – in the presence of exogenously added erythropoietin in marrow cultures. IL-9 also activates mast cell growth. **Interleukin 10 (IL-10)** has an role in the proliferation of the cytotoxic T-cells. It inhibits the synthesis of IFN-γ (by activated T-lymphocytes) and the production of IL-8 (by neutrophils). **Interleukin 11 (IL-11)** inhibits the formation of adipocytes and stimulates myelopoiesis in the long-term human bone marrow cultures. It also stimulates the formation of thrombocytes and possesses many of IL-6's effects [23]. **Interleukin 12 (IL-12)** stimulates proliferation of activated T-lymphocytes and NK-cells and induces synthesis of IFN-γ. **Interleukin 13 (IL-13)** possesses many of the biological properties of IL-4 and induces IFN-γ synthesis by the NK-cells [23].

Neopterin (NP) has been discovered for the first time in the bee larvae, working bee and royal jelly. The name of the substance comes from the Greek word "neo" meaning "new" and "pteros" – wing [14]. It's established that NP is a metabolite of GTP in the synthetic way of biopterin and that it is produced in significant quantities by monocytes and macrophages when stimulated with IFN-γ. Such a "producer" of NP is also the human monocyte cell lineage – THP-1 [27]. Monocytes and macrophages are the main source of NP compared to the other human blood cells [15] and the increased NP biosynthesis does not necessarily require activation of the T-cells (it can be caused by non-immune stimuli). W e i s s, M u r r [30], have shown that B-cells can also produce neopterin after stimulation with IFN-γ or IL-2.

W e r n e r et al. [31] show that during the *in vitro* treatment of human macrophages with IFN-γ, GTP-cyclohydrolase I increases its activity 7 to 40-fold which causes accumulation of NP and NP-phosphates. The immune response leads to a release of increased levels of NP (in serum, plasma, urine and other biological liquids) and this phenomenom is used as a biochemical monitoring of some diseases with pathological immune reactions [4, 14, 15]. NP serum levels above 10 nmol/l are considered high. B a i e r - B i t t e r l i c h et al. [3], show that the concentrations of NP correlate reversely with the absolute value of CD34+ T-cells and that they are significant predictors for the progression of AIDS. New physiological properties of NP – such as induction and stimulation of cell cytotoxicity and apoptosis, have been discovered in the recent years and its role as a chain breaking antioxidant has been proven [14, 17]. Experiments with H_2O_2 hemiluminescence indicate the potential role of neopterin and 7,8-dihydroneopterin free oxygen radical-mediated processes. It is established that *in vitro,* NP inhibits the expression of the gene for erythropoietin [1]. The exogenous administration of NP in cell cultures increases the number of CFU-GM and CFU-S which indicates that neopterin stimulates proliferation of hematopoietic stem cells [33 – 36; 1]. The affect of NP on stromal marrow cells has also been studied [34 - 36; 1], although the mechanisms of the functional interactions between the hematopoietic and stromal cells and different adhesion molecules participating in these interactions are unknown or partially identified. R o m a n i et al. [27] investigated dendritic cells as a possible "producer" of neopterin. It's known that the production of NP is stimulated by exog-

enously added LPS, while tumor-necrosis factor-α (TNF-α), IL-1β, IL-2, IL-10 and IL-18 are ineffective [32].

2. Nuclear factor kappa B - (NF-kB) affects on bone marrow hematopoiesis

For investigating the role of the different NF-kB proteins – p50, p52, p65 (RelA), taking part in the process of bone marrow cell proliferation/differentiation, it has been analyzed and compared the DNA-binding activity of all members of the NF-kB group in human monocytes (Mo), macrophages (Mac) and dendritic cells (DC). Nuclear extracts form blood Mo are used in the experiments with DNA-binding activity. Mainly p50/p50 homodimers are observed. The protein complexes binding with DNA and consisting of p50/RelB and p50/p65 increase and "additional complexes" of p65/p65 appearing during differentiation of Mo to Mac and terminal differentiation of immature DC - probably important for their biological activity [2]. The engagement of this proliferative factor in the stimulation of CD34+ marrow stem cells [35, 36], remains to be elucidated in further investigations.

3. Haematopoietic factors affecting erythropoiesis

Erythroid differentiation requires hematopoietic factors to proceed from early erythroid progenitors (burst-promoting units-erythroid – BFU-E) to mature red cells [28]. Numerous cytokines released from accessory cells have been shown to exert either stimulatory or inhibitory growth signals on the process of differentiation of mature erythrocytes. *In vitro,* erythroid cell growth also depends on variables such as type of culture medium, medium change schedule, temperature sera and the most widely used factors for *in vitro* stimulation of erythropoiesis [12].

Erythropoietin (Epo) is a 34 kDa glycoprotein produced by the kidney. This hormone is responsible for the regulation of erythropoiesis in mammals. It is established that committed erythroid progenitors (colony-forming unit-erythroid - CFU-E) have Epo receptors on their surface and require the presence of the hormone to survive *in vivo*, to proliferate *in vitro* and to activate the expression of erythroid-specific proteins, such as glycophorin and globin chains (10, 20). Dubart et al. [10] showed that Epo is also able to induce *in vitro* proliferation of pluripotent progenitor cells. In the presence of Epo alone, Epo-R virus-infected bone marrow cells gave rise to mixed colonies comprising erythrocytes, granulocytes, macrophages and megakaryocytes. Although other substances are now known to be required during the initial stages of erythropoiesis, late erythroid differentiation is regarded as strictly Epo-dependent [11]. Erythropoietin and stem cell factor (SCF) or insulin-like growth factor-I (IGF-I) were shown to act synergistically to promote proliferation and/or differentiation of erythroid progenitor cells and to prevent their apoptosis *in vitro* [29].

Interleukin 3 (IL-3) is required of differentiation of primitive erythroid progenitor cells (burst-forming unit, erythroid – BFU-E) mixed-cell colony-forming cells (CFU-Mix or CFU-GEMM) and spleen colony-forming cells (CFU-S) [20]. The presence of IL-3 in liquid cultures of CD34+ cells could be negative: this factor may inhibit long-term generation of CFC by inducing their maturation. The studies of Flores-Guzman et al. [12] show that the presence of IL-3 correlates with a decrease in erythropoiesis and an additional reduction in the number of myeloid CFC could be obtained. Attempts to stimulate erythropoiesis by addition of exogenous Epo have resulted in a further but also transient stimulation of CFU-E and erythroblasts. It is also known that

IL-3, even in the absence of serum and detectable Epo, is able to stimulate the full development of many erythroid bursts. This IL-3 effect is a dose-dependent and according to G o o d m a n et al. [13] does not appear to correlate with Epo dose. When addition of IL-3 or Epo to 7-day cultures was delayed, IL-3 but not Epo was shown to maintain BFU-E. **Interleukin 10 (IL-10)** with its synthesis-inhibiting effects on T cells and monocytes may be a potential candidate for indirectly affecting erythropoiesis. O e h l e r et al. [25] have observed that the addition of recombinant IL-10 to human PBMC cultures containing recombinant human Epo suppressed BFU-E growth in a dose-dependent manner at 10 ng/ml. In contrast, no inhibitory effect of IL-10 was seen when cultivating highly enriched CD34+ cells. Also, the addition of exogenous GM-CSF completely restored IL-10-induced suppression of BFU-E growth. This data indicates that IL-10 inhibits the growth of erythroid progenitor cells in vitro, most likely by suppression of endogenous GM-CSF production from T-cells. **Interleukin 11 (IL-11)** is a pleiotropic cytokine, isolated from a bone marrow stromal cell line and expressing activity on different stages of erythropoiesis *in vitro*: it sustains preferentially the terminal differentiation of the late erythroid progenitors CFU-E. The combination of IL-11 with the ligand for c-kit (KL) acts on early progenitors since it promotes the multiplication of pre-CFU-multi and stimulates the highly proliferative erythroid progenitors that yields remarkable erythroblast colonies in culture [26]. Addition of Epo to IL-11 induced the growth of erythroid progenitors (BFU-E) derived from CD34+ cells but not from the same population depleted of CD33(+)DR(+) cells. The combination of IL-11 with SCF, IL-3 or GM-CSF – in the presence of Epo, resulted in a synergistic or additive increase in the number of CFU cells. Moreover, the addition of SCF to IL-11 stimulated the development erythroid and multilineage colonies. **Interleukin 12 (IL-12)** has been classified along with Il-6, G-CSF, IL-11 and SCF as one of the biologically active substances supporting the growth of early progenitors in synergy with IL-3. It is not clear whether IL-12 directly influenced (inhibited) *in vivo* erythropoiesis or acted indirectly, by inducing the production of other hematopoietic growth factors. M o h a n et al. [21] observed the ability of IL-12 to directly enhance the expansion of erythroid progenitor cells from the bone marrow of normal and malaria-infected mice. They identify the cytokine as one of the factors playing a key role in the early up-regulation of red cell genesis. Moreover, the observation of higher levels of IL-12 in the sera of malaria-infected B6 mice also suggests its role for increased mobilization of erythroid progenitors – from bone marrow to extramedullary erythropoietic organs (spleen). IL-12 can also inhibit erythropoiesis by indirect mechanisms – most probably by its ability to induce production of haemopoietic cytokines (such as TNF-α and IFN-α) from accessory cells [21].

Thrombopoietin (TPO) is another factor that affects erythropoiesis. It has a synergistic effect with Epo or Epo plus IL-3 on erythropoiesis in human bone marrow: the addition of TPO to Epo significantly gave rise to more erythroid bursts and the addition of TPO to Epo+IL-3 might give rise to more erythroid bursts. TPO also has a synergistic effect with recombinant human G-CSF on the myelopoiesis in human bone marrow since the addition of TPO to G-CSF gave rise to significantly more granulocyte-macrophage colonies [18].

Bone morphogenetic proteins (BMPs) are pleiotropic regulators of tissue development and function, whose role in the control of haematopoiesis has not been extensively explored [9]. These secreated signalling peptides are members of the transforming growth factor- β (TGF-β) superfamily. The isoforms of TGF-β regulate the development of haematopoietic progenitors. Recent studies indicate that BMPs are autocrine/paracrine/hormonal regulators of haematopoietic development.

Activin A – a member of the TGF-β superfamily, promotes erythroid differentiation by stimulating the proliferation and differentiation of IL-3-responsive erythroid

BFU /while inhibiting the proliferation of IL-3-responsive granulocyte-monocyte colony forming units/ [9].

Stem cell factor (SCF) has a CFU-GM-enhancing and erythroid (BFU-E) potentiating activity on mouse, rat and human bone marrow. The experimental data indicate that the primary hematopoietic target cells and particular those with erythroid differentiation potential (BFU-E and CFU-GEMM) are primitive precursor cells. It is also a potent cofactor: at lowered concentrations and in combination with maximally acting concentrations of IL-3 or GM-CSF, it has additive activity on CFU-GEMM [6]. SCF and Epo can overcome the inhibitory effect of IFN-γ on erythroid progenitor cells in vitro, and high doses of Epo can correct the anemia associated with chronic disease – ACD [29].

Hydrocortisone – in both physiological (10^{-7} mol/l) and pharmacological (10^{-6} and 10^{-5} mol/l) concentrations, stimulated erythroid burst formation. In the concentration range of 10^{-7} to 10^{-5} mol/l this factor also appeared to increase erythroid colony size. Thus, it is suggested that the hormone may play a role in the physiological regulation of human erythropoiesis [16].

Inflammatory cytokines such as TNF-α and IL-6 play an important role in decreased erythropoiesis in patients with anemia of chronic disease – ACD [29] and rheumatoid arthritis (RA). Modulation of quantities of bone marrow erythroid progenitors during chronic inflammation may be one of the pathogenetic mechanisms leading to ACD.

Interferons are biologically active molecules produced by activated T lymphocytes and natural killer cells in response to viral infections as well as to a number of other stimuli. Although initially recognized and defined by their antiviral properties, it has become obvious that this group of glycoproteins may play a significant role in cellular differentiation, proliferation, and immunoregulation. IFNs have been shown to suppress the proliferation and/or differentiation of normal human bone marrow pluripotent (CFU-GEMM), CFU-GM, CFU-E, BFU-E *in vitro*. *In vitro* suppression of murine bone marrow megakaryocyte colony-forming cells has also been reported. Coutinho et al. [8] showed that the inhibitory effect of IFN-γ is most marked in the population of more primitive cells than the committed colony- and cluster-forming cells. It is determined that IFN-γ inhibits IL-6-dependent human myeloma cell growth and downregulates IL-6 receptor expression on the cell membranes as well as the mRNA synthesis. Ruhl et al [29] showed that IFN-γ inhibited the IL-6 induction of IL-4 receptors on the surface of the murine myeloid leukaemia M1 cell line, consistent with the myelosuppressive capacity of IFN-γ. More recently, the possibility that IFNs may play a role in the pathogenesis of aplastic anemia has been suggested [19, 29] and that one of the factors known to inhibit erythropoiesis is **IFN-γ**. Because blood IFN-γ levels are elevated and vary directly with the degree of the anemia in patients with hematologic malignancies and human immunodeficiency virus seropositivity and because IFN-γ directly inhibits erythroid progenitor colony formation, this cell factor appears to have a prominent role in the producing the anemia associated with chronic disease – ACD [29]. According to Mamus et al. [19], the inhibitory action of IFN-γ on erythropoiesis is mediated, at least in part, through Mo and T cells; an removal of these cells – before the addition of IFN-γ *in vitro,* significantly reduced its the suppressive effect. The authors also showed that CFU-E exhibited a greater sensitivity to IFN-γ (especially at lower doses), than BFU-E. The primary effect of this interferon may not be direct against BFU-E and CFU-E, but rather through changes in the activity of the accessory cell populations. The same authors [19] present data indicating that increased production of endogenous interferons may also represent a pathogenic mechanism for the development of pancytopenia in some patients with aplastic anemia.

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