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REVIEW ARTICLE

Haemopoietic growth factors; a brief review

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Circulating blood cells in humans originate from a common pool of multipotential haemopoietic stem cells found in the bone marrow. Multiple steps of cell division, differentiation and maturation are necessary before mature effector cells are released into the circulation. Haemopoietic growth factors stimulate the proliferation of progenitor cells being essential for their survival and contribute to the activation of mature cell function. In this review article we are dealing with some haemopoietic growth factors including stem cell factor (SCF), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage stimulating factor (M-CSF), granulocyte stimulating factor (G-CSF), and interleukin-3 (IL-3). Some of their biochemical characteristics, their physiological role on the blood cell progenitors, their involvement in the genesis of certain diseases as well as their newly presented therapeutic use are discussed in brief. *Hippokratia 2004, 8 (2): 88-92*

In adult life all circulating blood cells originate from a common pool of multipotential haemopoietic stem cells found in the bone marrow. Multiple steps of cell division, differentiation and maturation are necessary before mature effector cells are released into the circulation. A series of humoral factors known as haemopoietic growth factors, stimulate the proliferation of progenitor cells and are essential for their survival. Haemopoietic growth factors (HaemGFs) also contribute to the activation of mature cell function.

This review on HaemGFs considers Stem Cell Factor (SCF), Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Macrophage Colony Stimulating Factor (M-CSF), Granulocyte Colony Stimulating Factor (G-CSF), and Interleukin-3 (IL-3). Other factors such as Erythropoietin, Interleukins (ILs) IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-11, and Leukemia Inhibitory Factor (LIF) may also modulate haemopoeisis directly or indirectly, but are beyond the scope of this article.

The HaemGFs are glycoproteins with a varying carbohydrate chain, which is not essential for their biological activity. They are active in picomolar concentrations and exert their actions in a paracrine fashion. Both HaemGFs and their receptors show considerable structural homology suggesting that they are derived from a smaller repertoire of factors. HaemGFs differ as to their target cells both in lineage and in maturity but there is considerable overlap and some redundancy, the relevance of which remains unclear. Signal transduction pathways affected by HaemGFs and their receptors are currently the subject of intensive research. Genes for several of them have been cloned and their production engineered by recombinant technology has made them widely available. GM-CSF and G-CSF are used to accelerate marrow recovery after marrow transplantation, while G-CSF is used to support patients in the aftermath of chemotherapy and in treatment of chronic neutropenia.

Haemopoietic Growth Factors

I. Stem Cell Factor

Stem Cell Factor (SCF) is produced by a wide variety of cells including mast cells, eosinophils, macrophages, endothelial cells, and bone marrow stroma cells. It is an extensively glycosylated protein and is physiologically active as both a soluble 165- amino acid polypeptide and a membrane bound molecule. Transcription of SCF is potentiated by glucocorticoids and Interleukin-1b[1]. Its receptor c-kit protooncogene is a receptor protein tyrosine kinase broadly expressed on mature mast cells and eosinophils[2]. SCF promotes recruitment of mast cell progenitors into tissues, as well as their localization and maturation. It also enhances mediators' release from mast cells such as histamine, leukotrienes, GM-CSF, IL-5 and is required for IL-4 production by the mast cells. Additionally, in concert with IgE it sensitizes chemokine receptors in mast cells[3]. SCF induces mast cell growth and differentiation by activating signal transducer and activator transcription 5', which is a critical regulator of mast cell development and survival[4]. Bone marrow derived CD34+ cells stimulated by CSF result in production of mast cell colonies in vitro. In the co-presence of IL-9 there was an increase in both size and number of clonal mast cell cultures[5]. Synergism between SCF and GM-CSF has been shown to be essential for haemopoietic cell proliferation. The molecular mechanism of interaction was analyzed proving that SCF enhances the production of c-myc and cyclin-D3[6]. On the erythroid cell lineage SCF shows a synergistic effect with erythropoietin mediated

by the signal transducer and activator of transcription 5'[7]. SCF supports proliferation and survival of early haemopoietic cells by binding to the c-kit receptor resulting in prevention of apoptosis and also up regulates Bcl-2 and bcl-XL also in erythroid precursors (proerythroblasts), thus, protecting them from cell death[8]. This cytokine also plays a significant role in the proliferation of human megakaryotic progenitor cells. In the presence of thrombopoietin, the combination of erythropoietin, CSF and IL-9 increased the size of megakaryotic colonies[9]. Furthermore, SCF together with IL-15 and Flt-3 ligand can induce the differentiation of NK T-cells from human cord blood CD34+ cells[10]. Finally, recent experiments have shown that mesenchymal progenitor cells in the presence of SCF and IL-3 resulted in the generation of bone precursors expressing bone specific genes[11]. In humans, SCF alone or in combination with cyclophosphamide is utilized for the mobilization of haemopoietic and progenitor cells from the bone marrow.

II. Interleukin-3

Interleukin-3 (IL-3) is a cytokine produced by T-cells and mast cells. A disulphide bridge stabilizes the tertiary structure of this 15-30 kDa glycoprotein. IL-3 gene is composed of five exons and four introns. IL-3, IL-5, and GM-CSF share receptors, which are members of the haemopoietin receptor superfamily by a specific α chain and a common β chain shared among these cytokines for signaling. Acting in a similar way to GM-CSF and IL-5, IL-3 also contributes to the differentiation and function of leukocyte subpopulations[12]. IL-3 is a cytokine involved in stem cell survival and proliferation exerting its effect in both myeloid progenitor and haemopoietic stem cell populations. Stem cell survival is mediated by up-regulating the expression of bcl-2 and related genes[13]. On the other hand, IL-3 induces its proliferative and differentiative response through Src-Homology Protein tyrosine kinase-2 (SHP-2) found in wild type bone marrow progenitor cells[14].

IL-3 in cooperation with TGF-β induces differentiation of CD34+ progenitor cells toward Langerhans' cell development[15]. Multipotent progenitor cells undergo self-renewal in response to IL-3, displaying striking complexity including gene expression associated with different lineage subpopulations[16] IL-3 plays an important role in local eosinophil activation through IL-3Ra receptor found on the membrane of the eosinophils. Presence of IL-3 increases IL-3Ra expression. Recently conducted experiments showed IL-3 effect on basophil maturation. Collected peripheral blood stem cells, initially mobilized by G-CSF were cultured in the presence of IL-3 and after three weeks 20.0-83.3% of them were metachromatic. Peripheral blood stem cells derived basophils expanded in vitro were morphologically and functionally mature[17]. Although IL-3 is a cytokine involved in stem cell survival, differentiation and function, its physiological role remains obscure.

III. Granulocyte Colony Stimulating Factor

A Granulocyte Colony Stimulating Factor (G-CSF) activity was first discovered as the ability of serum from endotoxin treated mice, to induce differentiation of murine myelomonocytic cell line. The G-CSF gene products are 174 and 177 amino acid polypeptides with the larger being less active. Two internal disulphide bridges maintain the tertiary structure of this cytokine and are necessary for biological activity. G-CSF is synthesized by monocytes, endothelial cells, fibroblasts and mesothelial cells. It regulates both basal and neutrophilic production, stimulating the proliferation of bone marrow granulocytic progenitor cells and promoting their differentiation to granulocytes. G-CSF is the main enhancer of neutrophilic maturation in the late phase of development. G-CSF binds to G-CSF receptor, which in turn activates glycoprotein gp130. gp130 is the extracellular domain of the G-CSF receptor and its activation leads to the phosphorylation of Signal Transducer and Activator of Transcription-3 (STAT-3)-a crucial mediator for granulopoiesis. Suppressor of cytokine signaling-3 inactivates gp130, thus, negatively regulating granulopoiesis[18,19]. The expression of the receptors that mediate G-CSF effects on macrophages and neutrophils, is regulated by bacterial products, endogenous G-CSF, and cytokines, accounting for variable effects on neutrophilic function. This cytokine causes mobilization of neutrophils from the bone marrow. CD26, an extra cellular peptidase, is essential for G-CSF induced granulocyte mobilization. Additionally, G-CSF exerts a preventive role in neutrophilic apoptosis. Its depletion induces cell death of G-CSF dependent cell lines. The cytokine is degraded upon exposure to human neutrophil elastase and this has a negative effect on its ability to promote the in vitro proliferation and maturation of CD34+ cells. Neutrophil elastase was also found to decrease cell viability[20]. In humans, G-CSF is used together with chemotherapy in order to accelerate haemopoietic activity. Recombinant human G-CSF has consistently decreased the duration of neutropenia during all cycles of chemotherapy. Exogenous administration of G-CSF has found an extensive use in treating febrile neutropenias and congenital neutropenias[21]. At this point it has to be noted that in healthy humans G-CSF is not detectable in circulation but in infectious states circulating G-CSF can be measured; this fact underlines the notion that G-CSF is an emergency signal for neutrophil production under stressful situations. The ability of G-CSF to improve the function of both neutrophils and macrophages (in synergy with GM-CSF, IL-3 etc.) provides a rational for G-CSF therapy in non-neutropenic critically ill patients. Unique combination of haemopoietic anti-inflammatory and anti-infectious effects on the innate immune system also prevents from infection during major surgery[22]. Finally, it has been proved that recombinant G-CSF administration in partially hepatectomized mice accelerates liver cell proliferation and ameliorates injured liver cells regeneration[23].

IV. Granulocyte Macrophage Colony Stimulating Factor

The Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) DNA sequence codes for a 127-amino acid polypeptide with a predicted molecular weight of 14 kDa. The molecular weight of the native human GM-CSF ranges between 14 and 35 kDa depending on the degree of glycosylation. Endogenous GM-CSF is produced by fibroblasts (gingival, lung tissue cells etc.) and endothelial cells stimulated by IL-1αβ, TNF-α, and activated mononuclear peripheral blood cells. IL-10 release up-regulates the GM-CSF expression[24], while activated eosinophils release small amounts of GM-CSF acting in an autocrine manner in order to maintain its antiapoptotic effect[25]. As a multilineage haemapoietin this cytokine acts on the intermediate progenitor level and supports the proliferation of neutrophil, macrophage, and eosinophil colonies. Several laboratories have developed culture systems that allow the generation of human macrophages from monocytes using GM-CSF alone. Co-administration of GM-CSF and IL-4 in the culture medium expanded and matured the monocyte population into dendritic cells. These effects correlate with antigen presenting activity providing a mechanism by which systemic GM-CSF and IL-4 administration activates immunity in vivo[26]. Interestingly, GM-CSF incubated with human umbilical cord CD34+ and CD14+ stem cells were induced to differentiate into dendritic cells mainly in the presence of CD80 and CD88[27]. Although GM-CSF is best viewed as a major regulator of granulocyte and macrophage maturation, there is recent evidence that it plays a key role in allergic and autoimmune diseases. Allergens can induce GM-CSF production presenting an etiological argument for the role of the cytokine in allergic sensitization[28]. The interplay between dendritic cells and mediators is critical to the establishment of allergic airway inflammation. GM-CSF delays eosinophil apoptosis, thus, playing an important role in prolongment of the allergic response. In addition to its role in allergic reaction, GM-CSF also stimulates human neutrophils via activation of ERK (Extra Cellular Signal Regulated Kinase) and MAPK (Mitogen Activated Protein Kinase) resulting in increased superoxide release and adherence[29]. Furthermore, it elicits neutrophilic chemotaxis and chemokinesis, enhances neutrophilic recovery, increases class II MHC expression[30], facilitates antitumoral activity, and prevents infection acting as adjuvant vaccine agent[31]. Recently, it has been proved that intrahepatic cholangiocarcinomas with neutrophilic infiltration express frequently and intensively GM-CSF, the latter serving as a marker showing prominent neutrophilic infiltration and, thus, poor prognosis[32]. Nowadays, GM-CSF use, when given in humans, targets mainly to induce durable multilineage responses to a subset of individuals with bone marrow failure and to prevent neutropenia and febrile neutropenia in patients with malignant lymphomas[33].

V. Macrophage Colony Stimulating Factor

Macrophage colony stimulating factor (M-CSF) exceeds its effects on bone marrow progenitor cells. Three DNA sequences have been identified: M-CSFα encodes a 256-amino acid polypeptide, M-CSFβ a 554-amino acid polypeptide, and M-CSFγ consists of a 438-amino acid polypeptide. All three forms are glycosylated disulphide-linked homodimers. The first 150 N-terminal amino acids are needed to exert M-CSF biological activities. M-CSF is a potent survival and mitogenic factor for monocytes. In the presence of M-CSF monocytic colonies are stimulated towards macrophage production. Macrophage response is mediated through an ERK (Extra Cellular Signal Regulated Kinase) which is the key element involved in macrophage proliferation. M-CSF also regulates the differentiation of cells belonging to the monocytic lineage via various pathways one of which is the rapid catalytic activation of PCK-δ kinase found on the membrane of progenitor cells. Protein kinase-X associated as well with macrophage differentiation, is induced by M-CSF and PCK-δ[34]. M-CSF promotes monocyte survival through a Posphatidyl-Inositol-3-Kinase-dependent pathway, resulting in the phosphorylation of Protein Kinase B/Akt and the suppression of activation of Caspase-3[35]. Furthermore, M-CSF and osteoclast differentiation factor regulate osteogenesis in vivo. Osteoclasts differentiate from haemopoietic precursors of the monocyte/macrophage lineage in the presence of M-CSF and receptor activator of NF-kappaB ligand[36,37]. Since osteoclasts play an important role in pathogenesis of focal bone erosion in arthritis, further investigation is needed to examine the possible role of M-CSF in bone erosion in vivo[38]. Additional studies conducted in vitro demonstrated that M-CSF in cooperation with TGF-β1 could induce Langerhan cell development from haemopoietic progenitor cells in the absence of GM-CSF[39]. Finally it has been established that in patients with malignancies of the ovary as well as in patients with squamous cell carcinomas of the head and neck M-CSF serum levels are significantly higher compared to those of healthy individuals. Therefore M-CSF may act as a biological marker for these cancers[40]. When given in humans, M-CSF renders blood monocytes more effective by increasing the respiratory burst and monocyte migration into sites of inflammation.

Περίληψη

Δ. Μαρίτση, Α. Χαραλαμπόπουλος, Κ. Χαραλαμπόπουλος. **Αιμοποιητικοί αυξητικοί παράγοντες: Βραχεία ανασκόπηση.** *Ιπποκράτεια 2004 8(2) 88-92.*

Τα κυκλοφορούντα κύτταρα του αίματος στον άνθρωπο προέρχονται από μια δεξαμενή πολυδύναμων αρχέγονων αιμοποιητικών κυττάρων που εντοπίζονται στο μυελό των οστών. Πολλαπλές διαδικασίες που αφορούν στη κυτταρική διαίρεση και στη κυτταρική δια-

φοροποίηση καθώς και στην ωρίμαση αυτών των κυττάρων, απαιτούνται προτού οι ώριμες μορφές τους απελευθερωθούν ολοκληρωμένες στη περιφέρεια αποτελώντας κυτταρικά στοιχεία του αίματος. Οι αιμοποιητικοί παράγοντες αύξησης είναι απαραίτητοι στον πολλαπλασιασμό των προγεννητόρων κυττάρων, απαραίτητοι στην επιβίωσή τους και συνεισφέρουν στις λειτουργίες ενεργοποίησης των ώριμων κυτταρικών στοιχείων. Στο παρόν άρθρο ανασκόπησης γίνεται σύντομη αναφορά στον παράγοντα των αρχέγονων κυττάρων (Stem Cell Factor, SCF), στον παράγοντα τον διεγείροντα τις αποικίες των μακροφάγων κοκκιοκυττάρων (Granulocyte Macrophage Colony Stimulating Factor, GM-CSF), στον παράγοντα τον διεγείροντα τις αποικίες των μακροφάγων (Macrophage Colony Stimulating Factor, M-CSF), στον παράγοντα τον διεγείροντα τις αποικίες των κοκκιοκυττάρων (Granulocyte Colony Stimulating Factor, G-CSF) και στην ιντερλευκίνη-3 (IL-3). Παρέχονται στοιχεία σχετικά με τα βιοχημικά χαρακτηριστικά τους και συζητείται με συντομία ο ρόλος τους από πλευράς φυσιολογίας, η συσχέτιση τους με ορισμένες παθήσεις καθώς και η χρήση τους ως θεραπευτικά μέσα.

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