REVIEW ARTICLE

Haemopoietic Growth Factors — From Discovery to Clinical Application

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Summary

The Colony Stimulating Factors (CSFs) are a family of haemopoietic hormones that likely share a common ancestral origin and stimulate white blood cell development. They display unique but overlapping biological functions and stimulate the survival, proliferation, differentiation and functional activation of granulocytes and monocytes/macrophages and their precursor cells *in vitro* and *in vivo*. Each hormone has been purified and produced in active recombinant form. Recombinant G-CSF and GM-CSF are now being used around the world in a variety of clinical situations (e.g., in conjunction with chemotherapy and bone marrow transplantation) to promote the formation and function of these leukocytes. These molecules are among the first of a new generation of biological agents that will impact enormously on clinical medicine.

Key words: Colony stimulating factors, cytokines, haemopoiesis.

Introduction

The mammalian haemopoietic system is composed of a number of different cell types, each with a specialised function. These cells are present in the circulation and resident in the haemopoietic organs, principally the bone marrow and spleen. Over 99% of circulating blood cells are erythrocytes. In addition, there are platelets (derived from megakaryocytes) that are important in thrombus formation and 6 different types of leukocytes. Mature circulating cells have a very short life span (from days to weeks) and dying cells are therefore required to be continually replenished. The new cells are generated from progenitor cells in the bone marrow and the rate of production is extremely high – over 10^{10} new cells are produced daily in an adult. The production and maintenance of steady-state haemopoietic systems can react specifically to environmental stress. Thus, reduced oxygen tension induces an increase in red blood cells, parasitic infection results in elevated eosinophils and bacterial infection precipitates an increase in neutrophils.

The Colony-stimulating Factors

The production of blood cells, both in response to specific stimuli and in the steady-state is controlled at least in part by a family of glycoprotein hormones or growth factors. The first identified haemopoietic hormone was erythropoietin – a specific stimulus for the generation of erythrocytes. This growth factor was originally described in 1906[1]. However, it proved much more difficult to identify equivalent growth factors for the myeloid cell lineages, and it was not until the development of *in vitro* culture systems for haemopoietic cells in the mid-1960s that a possible method emerged to define and characterise haemopoietic hormones active on other cell types[2]. The development of this culture system allowed the process of proliferation and differentiation of haemopoietic progenitor cells that occurs in the bone marrow to be mimicked *in vitro*. The generation of colonies of mature cell types from bone marrow progenitor cells in culture absolutely required the presence of soluble growth factors and the descriptive name of colony-stimulating factor (CSF) was applied.

During the 1970s these molecules were characterised in considerable detail and it became clear that at least 5 different molecules were capable of stimulating the growth of white blood cells. The purification of these molecules was extraordinarily difficult. This was due to both the minute amounts of CSF present in, or produced by, even the richest tissue source and the up to 1 million-fold purification that was required to achieve purified protein. Although the different CSF molecules are each biochemically unique they display distinct but overlapping spectra of biological activity. Thus, Granulocyte-CSF (G-CSF)[3] and Macrophage-CSF ((M-CSF)[4] stimulate primarily the growth of neutrophil-granulocyte and monocyte-macrophage colonies respectively. In contrast, Granulocyte-Macrophage-CSF (GM-CSF)[5] stimulates both of these colony types but at higher concentrations is also active on eosinophil, erythroid, megakaryocyte and multipotential progenitor cells. Multipotential-CSF (Multi-CSF or Interleukin-3)[6,7] has a broader spectrum of activities – it stimulates the cell types mentioned above but is also active on mast cells and some stem cells. Eosinophil-CSF (or Interleukin-5) is active on eosinophils and B-lymphocytes[8].

The most dramatic advance in the development of the CSFs occurred with the introduction of molecular biology into the field. By the early 1980s small quantities (a few μg) of the CSFs had been purified. From the potency of their biological actions *in vitro*, their clinical potential in stimulating blood cell formation was obvious. However, the minute amounts of available CSFs precluded any clinical use. The utilisation of molecular biological techniques allowed the mass production of these hormones in micro-organisms (e.g., bacteria/yeast). Between 1983 to 1986, the genes for G-CSF, M-CSF, GM-CSF, Multi-CSF were molecularly identified and production of large amounts of protein commenced. This meant the efficacy of the CSFs could be tested in experimental animals and ultimately in man. The potency of these molecules in stimulating the production of leukocytes *in vitro* was recapitulated *in vivo* and their clinical potential has become a reality.

The Organisation of Haemopoiesis

As with other self-renewing systems, the haemopoietic system is arranged in an hierarchical manner. At the top of the family tree is a rare population of multipotential stem cells. These cells have enormous proliferative potential. They have the capacity to generate cells of all haemopoietic lineages, the ability to self-generate and can repopulate the haemopoietic system of an entire animal. The progeny of the stem cells retain the capacity to generate enormous numbers of daughter cells, but lose their ability to generate multiple lineages of cells – they become 'committed' to a restricted lineage (or lineages) and are therefore called committed progenitor cells. These are the cells that give rise to colonies in *in vitro* cultures, and their progeny pass through the familiar morphological stages recognised in bone marrow and culminating in mature differentiated cells.

The mechanisms that control this process are (i) the haemopoietic hormones (e.g., the CSFs) and (ii) stromal cells — specialised cells within the bone marrow microenvironment that regulate stem cell behaviour by direct cell-cell contact.

At least 16 haemopoietic regulator molecules have so far been identified[9] that are able to stimulate proliferation of haemopoietic cells; and a single precursor cell can respond to as many as 6 different regulators (see Table I). Many, therefore, have overlapping actions that can involve cells of different lineages or hierarchical subsets of cells. In addition to the obvious redundancy that exists in control of haemopoiesis, the situation is made even more complex by a complicated set of interactions that can occur as a result of stimulation by a CSF. For example, T-lymphocytes can be stimulated to produce M-CSF, GM-CSF or Multi-CSF (all of which can act on monocytes/macrophages) when activated by a variety of stimuli. As a result, a nearby macrophage can be activated to release IL-1 or gamma-interferon, both of which can modulate the function of the initiating T-lymphocyte. This complex interaction of cells and their regulators provides a sophisticated control network for regulation of haemopoiesis.

Table I
Haemopoietic regulator molecules

Haemopoietic Hormone	Acronym	Target Cells
Erythropoietin	Epo	E, Meg
Granulocyte-Macrophage CSF	GM-CSF	M, G, Eo, Meg, E, Multi
Granulocyte CSF	G-CSF	G, M
Macrophage CSF	M-CSF	M, G
Multipotential CSF	Multi-CSF/IL-3	M, G, Eo, Meg, E, Multi, Stem, Mast
Interleukin 1	IL-1	T, Stem
Interleukin 2	IL-2	T, B
Interleukin 4	IL-4	T, B, mast, GM
Interleukin 5	IL-5	EO, B
Interleukin 6	IL-6	B, G, Meg, Stem
Interleukin 7	IL-7	B, T
Interleukin 8*	IL-8	G
Interleukin 9	IL-9	T, Mast, E
Interleukin 10	IL-10	Mast, B
Interleukin 11	IL-11	M, Multi, B, Meg
Steel Factor (Stem-cell factor)	SF (CSF)	Stem, Multi, G, Mast
Leukemia Inhibitory factor	LIF	Meg, M

E=erythroid; M=macrophage; G=neutrophil granulocyte; Eo=eosinophil granulocyte; Mast=mast cell; Meg=megakaryocyte; Multi=multipotential progenitor cell; Stem=stem cell; T=T-lymphocyte; B=B-lymphocyte.
**IL-8 has been characterised as a chemotactic agent for neutrophils, T-cells and basophils (and can influence other functions such as neutrophil adhesion). It has not been demonstrated to stimulate cellular proliferation.*

Biological Actions of the CSFs *in vitro*

The CSFs were purified based on their ability to stimulate the proliferation of target cells *in vitro*. However, in addition to their ability to stimulate proliferation, these factors also influence differentiation and maturation of responding cells; they can irreversibly induce a bipotential granulocyte-macrophage progenitor cell to become committed to the generation of macrophage progeny, thus influencing irreversibly the genetic programme within that cell[10]. They can act in a similar manner on mouse and human leukemia cells to induce irreversible differentiation-commitment with subsequent extinction of the leukemic phenotype[11].

The CSFs also allow precursor cells and mature cells to survive *in vitro*[12] — they maintain intact the membrane transport system of cells for glucose, ATP and sodium/potassium. Furthermore, they stimulate the functional activation of mature cells (e.g., superoxide production, phagocytosis, chemotaxis, etc).

These actions of CSFs are mediated via specific receptors on the surface of cells, many of which exhibit striking homology in their extracellular domain. These receptors are now recognised to form a superfamily of growth factor receptors with links to both the immunoglobulin and interferon receptor families[13]. The homology between haemopoietic growth factor receptors is of interest because it provides an explanation for the similar biological effects of the growth factors despite their apparent structural dissimilarity at the amino acid level. Presumably, as information on the tertiary structure of the growth factors becomes available, similar receptor-binding domains will be recognised in previously apparently unrelated molecules.

Clinical Uses of Colony-stimulating Factors

G-CSF and GM-CSF are the first members of the haemopoietic growth factor family to be used around the world in a variety of clinical situations. The current clinical uses relate directly to their effect on haemopoiesis as defined *in vitro* but it is likely that additional uses for these hormones will be defined as understanding of their *in vivo* interactions increases. A number of additional factors are currently being assessed as to their potential clinical utility. The clinical use of these agents has recently been reviewed in detail[14-16].

Primary Bone Marrow Failure Disorders

One of the most dramatic effects of G-CSF is its ability to restore the neutrophil count to normal in children with rare congenital neutropenias (including cyclic neutropenias and Kostmanns Syndrome[17,18]). The children suffering from these disorders have recurrent episodes of oral, pharyngeal and gingival infections and are at high risk of developing bacteraemias and pneumonia. This is dramatically reversed with G-CSF. Acquired idiopathic neutropenia also responds to treatment with G-CSF. However, unfortunately, patients with severe aplastic anaemia do not consistently respond to treatment with G-CSF or GM-CSF.

Chemotherapy-induced Neutropenia

Cytotoxic chemotherapy is frequently associated with complications of neutropenia and thrombocytopenia. These are serious side-effects and are a major cause of morbidity and mortality in this context. The risk of serious infection has long been recognised to be related to both the severity and duration of the neutropenia. Both G-CSF and GM-CSF are able to minimise the neutropenia associated with chemotherapy when used prophylactically and this translates into a very significant reduction in infections and rates of hospitalisation[19-21]. G-CSF and GM-CSF will likely also have a role to play in treating infections in patients with chemotherapy-induced neutropenia.

This action of the CSFs to reduce chemotherapy-associated neutropenia allows studies to be performed investigating the effect of increasing doses of chemotherapy in an attempt to improve cure rates of malignancy. A number of such studies are currently underway.

Bone Marrow Transplantation

After bone marrow transplantation, there is a period of profound neutropenia and thrombocytopenia which lasts several weeks. Both G-CSF and GM-CSF have a striking effect in this situation and are able to induce accelerated myeloid engraftment. As a result, the duration of antibiotic therapy and hospitalisation is dramatically shortened[22]. As expected from the *in vitro* actions of both CSFs, platelet engraftment is not altered.

An unexpected action of the CSFs is their ability to stimulate the release of all types of progenitor cells (and possibly stem cells) from the bone marrow into the peripheral blood[23]. These cells can then be efficiently collected by leukopherisis and used to augment bone marrow transplantation[24]. G-CSF has therefore been used to stimulate the release of progenitor cells; cells were collected and reinfused after high-dose chemotherapy. As a result, platelet recovery was profoundly enhanced. In the first 17 patients treated in this manner, the time to recovery of a platelet count of $50x10^9/1$ was a median of 15 days compared with 39 days in controls, and the

need for platelet transfusion decreased accordingly[25]. In addition, neutrophil recovery remained rapid. These results suggest that use of growth factor mobilised peripheral blood progenitor cells may have a wide application in situations of high-dose chemotherapy and possibly in allotransplantation.

Myelodysplastic Syndromes and Leukemia

Myelodysplastic syndromes are a common and heterogeneous group of premalignant bone marrow disorders that cause varying degrees of anaemia, neutropenia and thrombocytopenia. Patients therefore suffer from recurrent infections, bleeding, and, in a proportion of patients, transformation to acute leukemia. In one randomised study, GM-CSF has been demonstrated to be of value in preventing infections in these patients[26]. In addition, there was no increase in the number of patients whose disease transformed to acute leukemia, despite the theoretical possibility that this could occur. GM-CSF may therefore provide a useful adjunct to other supportive therapy in this group of patients.

In vitro evidence clearly demonstrates the ability of CSFs to stimulate the proliferation of leukemic cells. In addition, they paradoxically stimulate differentiation (and ultimate suppression) of some leukemias. These agents have therefore been used cautiously in patients with leukemia. A randomised study of G-CSF in patients with relapsed acute leukemia has documented its ability to reduce infections following chemotherapy[27]. Studies are also underway to exploit the action of CSFs to stimulate leukemic cell proliferation and increase cell kill use of cell-cycle specific chemotherapy.

Infections and AIDS

It is likely that CSFs will find a role when used in combination with antibiotics for patients with established infections and normal neutrophil counts. However, this role remains to be established. The potential role of CSFs in patients with AIDS is actively being investigated. Treatment with zidovudine for example, is limited by its effects on bone marrow and thus treatment with CSFs might be expected to be of value.

Administration and Side-effects

The CSFs are polypeptide hormones and must therefore be administered parenterally. All CSFs have short half-lives when injected intravenously (1-3 hours) and more sustained levels can be obtained using the subcutaneous route. The dose of G-CSF is between 3-20 μg/kg/day and for GM-CSF is 5 μg/kg/day with a maximum dose of 10 μg/kg/day.

Side-effects of therapy are minimal. G-CSF is extremely well-tolerated, with the main side-effect being mild bone pain and, in children, asymptomatic splenomegaly. With GM-CSF there may be a dose effect (hypotension-hypoxia syndrome) and fever and rash may occur. It is possible that these effects are mediated by the release of additional molecules *in vivo.*

Future Haemopoietic Growth Factors

While G-CSF and GM-CSF are being widely used for the treatment of neutropenia, thrombocytopenia, with its consequent risk of haemorrhage, remains a major cause of morbidity and mortality. As a result, a number of potential agents active on megakaryocytes and their progenitors are actively being pursued and are likely to find their way into widespread clinical use over the next few years. Several promising growth factors in this regard include interleukin-6, interleukin-11 and Leukemia Inhibitory Factor (LIF). These molecules show very promising platelet activity in animals and are being developed for human studies.

Another area of active study involves the identification and characterisation of growth factors active on haemopoietic stem cells. It is easy to imagine that some factors would play a valuable role in bone marrow transplantation or in situations where cells are manipulated *in vitro* prior to reinfusion into the patient.

Thus, the CSFs are among the first in a new generation of biological agents that have the potential to impact enormously on clinical medicine.

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