

Leukocyte Growth Factors

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It has long been recognized that if neutrophil levels fall below $1,000/\mu\text{l}$, patients develop a serious risk of contracting major infections or have difficulty overcoming a range of bacterial infections, despite the use of antibiotics. The same problems arise if the functional activity of neutrophils is inadequate. No formal studies have been made on the importance of adequate numbers of monocytes or macrophages but it is likely that a similar situation applies. The most common hospital situation in which inadequate numbers or function of neutrophils or monocytes occurs is in cancer patients following chemotherapy or radiotherapy, with or without attempted hematopoietic cell transplantation. However, a similar situation is likely to occur as a complication of a wide range of diseases and with at least some community-acquired infections, not to mention infections occurring secondary to AIDS. There are therefore many clinical situations in which supportive therapy to patients would be helpful or life-saving if methods existed for stimulating an increase in neutrophil and monocyte numbers or their functional activity. Clinical agents are now available in the form of hematopoietic growth factors and these have already been widely used, but often in a manner that is suboptimal.

It is useful to briefly review the regulators controlling granulocyte and macrophage formation, their basic biology and their value in various clinical situations.

Regulatory Control of Granulocyte- Macrophage Formation

Formation of granulocytes and macrophages is continuous throughout life because these are short-lived cells. Most cell formation occurs in the bone marrow, with the spleen contributing small additional populations. Granulocyte and macrophage formation is a subset of the general process of blood cell formation in which cells of eight major lineages are produced by a small set of self-sustaining multipotential stem cells. This occurs in a stepwise process in which stem cells can each form up to at least 5,000 lineage-committed progenitor cells. These progenitor cells are not self-generating and expend themselves by each forming up to 10,000 mature cells. Once a cell has become a committed progenitor cell, for example in the granulocytic or macrophage lineage, it cannot switch into another lineage such as the erythroid, nor can it de-differentiate and again become a stem cell. Progenitor cells generate their mature progeny in a sequential manner. For example in the neutrophil lineage this sequence is: myeloblast→promyelocyte→myelocyte→metamyelocyte→neutrophil, a process in which cell division is coupled with increasing maturation until, at the last two stages, non-dividing but maturing progeny are produced.

Special signals in the form of chemokines are required to release these cells from the bone marrow to the blood, then attract them to various tissue locations, particularly to sites of infection or tissue damage.

The control of cell division and the initiation of maturation appear to involve both specialized local micro environmental cells in the marrow and a group of secreted regulatory molecules sometimes called cytokines, growth factors or regulatory factors. In reality, the two control systems involve closely similar processes because local microenvironmental cells produce many of the known regulatory factors. However, these cells are not the only source of such regulatory factors. It is one of the characteristic features of most hematopoietic regulatory factors that they can be produced by many cell types present in all organs (e.g. endothelial cells, fibroblasts, macrophages, lymphocytes and quite possibly most parenchymal cell types). Normally, production rates are low in good health but can rapidly be increased in emergencies, the most common of which for regulators of granulocytes and macrophages are inducing signals originating from microbial products or tissues damaged by microorganisms.

Thus a highly labile system exists in the body in which many cell types can quickly respond to the appearance of microorganisms by increasing regulator production either for local use or as a systemic humoral stimulus, the short half-life of the regulators ensuring a rapid return to basal levels when the inducing signals have been eliminated.

Figure 1 shows the major hematopoietic regulators controlling granulocyte and macrophage formation. By their action, increased numbers of maturing cells are produced by progenitor cells in a dose-dependent manner but simultaneously, stem cells are stimulated to form replacement or additional progenitor cells. In this manner, increased cell production can be sustained for days, weeks or months as the need arises without completely expending all progenitor cells.

From the pattern in **Figure 1** it is evident that many regulators can stimulate both progenitor and mature cell production and that multiple regulators exist that are able to stimulate the formation of particular types of blood cell. This is not a redundant control system. From gene inactivation studies (**Table 1**) it is now known that each regulator has certain unique functions not able to be compensated for by other regulators. It is also now known that different types of precursor cells have differing requirements for stimulation by regulators. For example, stem cells have a mandatory requirement for simultaneous stimulation by multiple regulatory factors. In contrast, progenitor cells can be stimulated quite effectively by single regulators. However, if com-

binations are used, resulting cell production can be greatly enhanced—so-called, superadditive synergistic responses. A further complexity regarding these regulators is that none exhibits strict lineage specificity in its action. All have actions on one or more additional lineages—a somewhat unexpected arrangement, but one possibly designed to coordinate the production of various types of blood cells. Most hematopoietic regulators are glycoproteins in the 18 to 40 kD size range. The carbohydrate moiety of the molecule is not required for biological action but can be vital in prolonging the half-life of the molecule in vivo. Typically, the polypeptide chain of the regulator comprises four α -helices, maintained in the correct conformation by disulfide

bonds. Each regulator contains two small active regions that bind specifically, and with high-affinity, to matching specific transmembrane receptors. The regulator needs to engage at least two receptor chains to form a high-affinity signaling complex, which is then activated by phosphorylation in the cytoplasmic domains of the receptor chains. The activated receptor complex then binds to and activates a variety of signaling molecules, eventually resulting in activation of various genes in the responding cell.

A notable feature of these regulators is that they are not simply growth factors but also have other actions on responding cells (1). These include: maintenance of viability and membrane transport integrity (regulator-deprived cells die by apoptosis), controlling certain lineage commitment decisions, initiating maturation and, not least, activating the functional activity of the mature cells eventually produced. The various signals required to elicit these different cellular responses emanate from different regions of the receptor chains. An important consequence of this economical control arrangement is that, when regulators are used to increase cell production, they also can have highly important actions on the functional capacity of the mature neutrophils and macrophages produced. Thus the therapeutic use of such regulators enjoys a bonus in achieving not only more cells but also cells with activated functional activity.

Hematopoietic regulators cannot be extracted in sufficient amounts from tissues or cells to be usable clinically. The molecules are also too large and complex to be synthesized. Therefore, all regulators in extensive experimental use or in clinical use are recombinant molecules, mass-produced by inserting the regulator gene into suitable bacterial, yeast or mammalian cell expression systems.

All of the human-active regulators shown in Figure 1

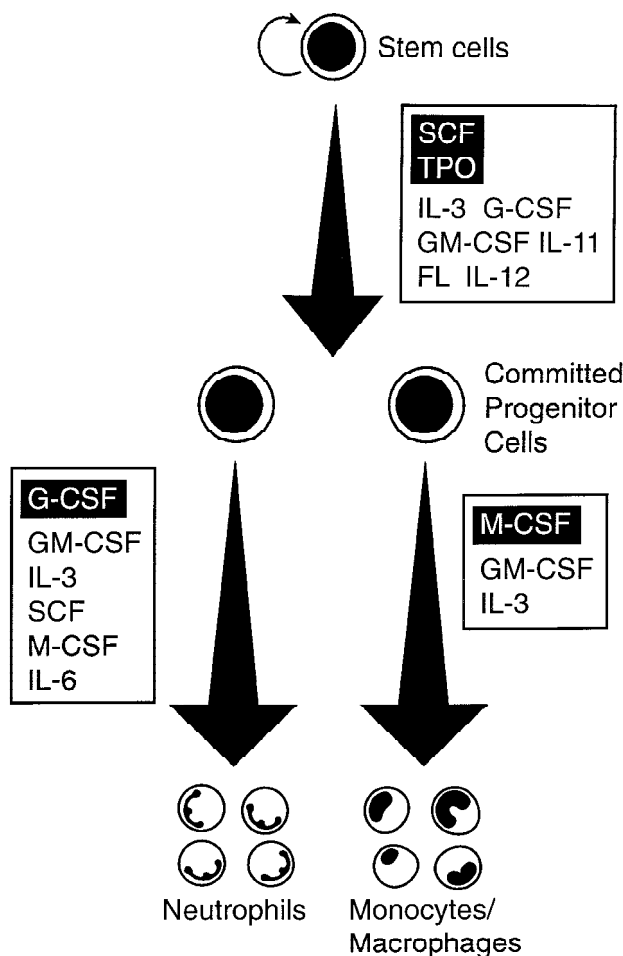


Figure 1. The production of mature neutrophils and monocytes occurs in two stages. The diagram shows the regulatory factors known to be active in stimulating the two stages. Highlighted receptors have been shown by gene inactivation experiments to be of greater quantitative importance. SCF = stem cell factor, TPO = thrombopoietin, IL-3 = interleukin-3, G-CSF = granulocyte colony stimulating factor, GM-CSF = granulocyte-macrophage colony stimulating factor, IL-11 = interleukin-11, FL = flt-3-ligand, IL-12 = interleukin-12, M-CSF = macrophage colony stimulating factor, IL-6 = interleukin-6.

Table 1. Consequences of Inactivation of Genes Encoding Hematopoietic Regulators

Regulator	Consequences
G-CSF	Severe neutropenia, defective responses to infections
GM-CSF	Alveolar proteinosis, defective lung macrophages, immune responses and responses to infections
M-CSF	Severe reduction in macrophages and osteoclasts, osteopetrosis
IL-3	Defective responses to helminth infections
SCF	Depleted stem cells, anemia
TPO	Severe thrombocytopenia, depleted stem cells
IL-5	Severe reduction in eosinophils
EPO	Failure of erythropoiesis, fetal death
LIF	Failure of blastocyst implantation
IL-11	Defective placental development, fetal death

G-CSF = granulocyte colony stimulating factor; GM-CSF = granulocyte-macrophage colony stimulating factor; M-CSF = macrophage colony stimulating factor; IL-3 = interleukin-3 (Multi-CSF); SCF = stem cell factor; TPO = thrombopoietin; IL-5 = interleukin-5; EPO = erythropoietin; LIF = leukemia inhibitory factor; IL-11 = interleukin-11.

have been mass-produced in recombinant form and are potentially available for clinical use. However, before being able to be used in the clinic, such products need to be tested as single agents and shown to be both efficacious and without significant side effects. This rather inflexible testing presents two problems: (a) A regulator may need to act in association with another before being obviously active. Such agents will be declared inactive in the present test procedures. (b) Because regulators act on multiple cell lineages and stimulate mature cell function, side effects may result from their action. If obvious, this will tend to make a regulator unacceptable for licensing. It might be supposed that the normal body regulators would uniformly lack toxic side effects but this is not so, most often because of the stimulation of mature cells to release potentially toxic products. Partially as a consequence of this rather rigid testing protocol, few of the potentially useful regulators have in fact been licensed for clinical use.

In the broad range of hematopoietic regulators, only five have been licensed for clinical use—IL-2 for T-lymphocytes, erythropoietin for erythropoiesis, IL-11 for stimulation of platelet formation, G-CSF and GM-CSF for stimulation of granulocyte and macrophage formation (2,3) and SCF to enhance CSF responses.

In some contexts, G-CSF and GM-CSF have an equivalent action because both are proliferative stimuli for granulocytes. However, the action of G-CSF is clearly stronger than that of GM-CSF when assessed merely by the levels of granulocytes achieved in the blood. GM-CSF has a broader action, stimulating in addition monocyte and eosinophil formation. In addition, from gene deletion studies, GM-CSF is now known to have a powerful effect on macrophage function, at least in lung tissue, and a special action in activating

the dendritic cells that are necessary to process and present antigens to T-lymphocytes to initiate many immune responses.

In terms of resistance to particular types of infection, G-CSF might be expected to be more effective in increasing resistance to bacterial infections, while GM-CSF would be most effective in increasing resistance requiring macrophage activation as may be the case for fungal infections.

Clinical Applications

Unfortunately, the CSFs need to be administered by injection and because of their short half-life of a few hours, are most effective when given subcutaneously once or twice daily. Both G-CSF and GM-CSF have been used effectively in patients receiving chemotherapy as agents able to accelerate regeneration of neutrophil levels and to reduce somewhat the necessity for antibiotic use or extended hospitalization (Figure 2). Similarly, both agents are effective in shortening recovery times of neutrophils after bone marrow transplantation (2,3). Less detailed information is available for their respective effectiveness in preventing or truncating infections in other situations.

However, there are two uncommon disease states, cyclic neutropenia and congenital neutropenia, in which the daily injection of CSF has allowed patients to achieve normal levels of neutrophils and then to exhibit normal resistance to incidental infections. In the case of congenital neutropenias where G-CSF is required (4), this action has proved life-saving, although a small proportion of these patients subsequently develop acute myeloid leukemia. This is unlikely to be due to the proliferative effects of G-CSF because simple overstimulation of cell division does not lead to leukemia development (5). It is more likely that this subset of patients is in fact in a preneoplastic state prior to treatment. An unanticipated effect of CSF treatment was the development of major rises in stem and progenitor cell numbers in the peripheral blood. In this action, G-CSF elevates levels to a higher degree than does GM-CSF. These peripheral blood stem cells (PBSC) have proved superior to marrow cells because they result in faster recovery of neutrophil and platelet levels following transplantation (6). The self-administration of CSF by patients with harvesting of such peripheral blood stem cells by apheresis has largely replaced harvesting of bone marrow as the cell population of choice for transplantation, largely due to the relative ease of the procedure and the larger cell yields possible. This day-care approach to transplantation is having a major impact on clinical procedures where high-dose chemotherapy is being used in the management of cancer patients, although in this setting, techniques to separate hematopoietic stem cells from contaminating circulating tumor cells are desirable.

A final clinical use of G-CSF injected into volunteers is to elicit large numbers of mature neutrophils in the peripheral blood which can then be collected and infused into severely neutropenic patients as an emergency procedure.

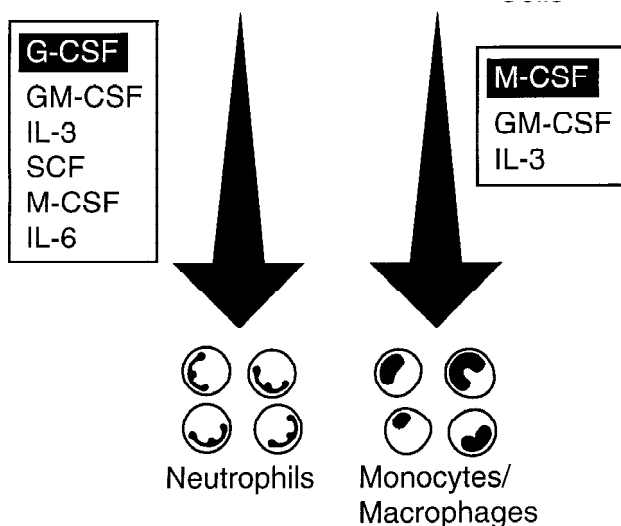


Figure 2. Diagram showing the current clinical uses of G-CSF and GM-CSF. It is unclear at present whether the enhanced wound healing induced by GM-CSF is due to actions on macrophages, dendritic cells or skin epithelial cells.

Comments

The use of G-CSF and GM-CSF is still in an early phase despite their clinical use for more than a decade in many hundreds of thousands of patients. There are several reasons for expecting that the clinical results from the use of these agents should be able to be improved. First, the body uses *combinations* of regulators, not single regulators, as in present clinical medicine. Combinations are more effective, should have fewer side effects and should eventually prove cheaper when pharmaceutical rationalization becomes a reality. Granulocytes and macrophages work as a team in their antimicrobial actions and it makes little sense only to stimulate one or other cell population when attempting to enhance resistance. Second, all experimental models show that the use of CSFs *before* the establishment of infections or leukopenia is more effective in enhancing resistance to infections rather than, as in present clinical use, the administration of CSFs *after* the onset of neutropenia. Where chemotherapy is planned, such pretreatment is technically feasible and it needs more extensive clinical trials than those so far undertaken to establish whether humans resemble animals in the importance of timing of CSF treatment for increasing protection against infections. Third, the special roles played by GM-CSF in fungal infections, in accelerating wound healing and in enhancing immune responses have all been documented experimentally and been the subject of minor clinical reports but have not had extensive clinical trials (3). This is overdue.

Overall, the CSFs have justified their development even with their present, rather illogical, manner of use. Clinical results must be improved by a more intelligent mimicking of the methods used in the body. Additional recombinant agents such as M-CSF, IL-3 or SCF are available but, when tested alone, have been rejected either because of inadequate efficacy or side effects. It is to be hoped that methods will be devised to check the effectiveness of the many possible combinations in low-dose regimens of growth factors where there is likely to be little risk of side effects.

When this is accomplished, I anticipate a much different pattern of regulator use from that occurring at present in the clinic.

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