Mechanisms of Disease

Colony-stimulating factors in the pathogenesis and treatment of disease

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Clinical trials of colony-stimulating factors in the treatment of various haematological and infectious diseases have been initiated. This review summarizes the recent evidence from studies in vitro and in vivo which provide support for the role of these haemopoietic growth factors in the pathogenesis and potential therapy of such conditions.

The production of haemopoietic cells is dependent on the sequential actions of glycoprotein growth factors at all stages from the multipotent stem cell in the fetal liver or adult bone marrow to the mature cell in the periphery. The characterization of these growth factors over the past 20 years followed the initial observation that the growth of single haemopoietic progenitor cells to form colonies in vitro depends on the presence of exogenous stimuli, hence the generic term 'colony-stimulating factor' (CSF). The CSFs induce the proliferation of progenitor cells and influence their progressive commitment to various differentiation pathways, and they have been classified according to the specific lineages on which they exhibit their most obvious effect. For example, erythropoietin (Epo), perhaps the haemopoietic growth factor most familiar to clinicians, acts relatively late in the erythroid differentiation pathway, stimulating both proliferation and haemoglobinization. However, this review will be restricted to a discussion of the non-erythroid CSFs which include multi-potential CSF (Multi-CSF, interleukin 3), granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF) and eosinophil CSF (Eo-CSF, interleukin 5) (Figure 1). Recently the use of recombinant DNA technology has allowed molecular cloning of the genes encoding most of these factors, their expression in bacteria, yeast or mammalian cells, and the production of purified material in high yield for further laboratory investigations and clinical trials.

Structure

The murine and human CSFs which have been characterized are glycoproteins and all but the M-CSFs are single-chain polypeptides containing intra-chain disulphide bridges which are essential for biological activity. Human and murine M-CSF are disulphide-linked homodimers. There is no sequence homology between the different polypeptides within a species but the variable homology which exists between members of the same class in different species is reflected in the activity of human G-CSF and M-CSF on murine cells and that of murine G-CSF and Eo-CSF on human cells.10

The CSFs interact with specific high-affinity receptors which are present in relatively low numbers on the surface of normal target cells, and they exhibit their biological activity at picomolar concentrations in vitro.11 The carbohydrate portion does not appear to be necessary for receptor binding or biological action and possibly has some other function in vivo. However, studies using murine Multi-CSF and GM-CSF did not indicate that glycosylation has a significant influence on the rapid clearance of these molecules from the circulation.

Production

The human cellular sources of CSFs include activated T lymphocytes, macrophages, fibroblasts and endothelial cells. Neutrophilia is also observed in some patients with CSF-secreting solid tumours12 and several tumour cell lines have been used for purification of CSFs on account of their aberrant high production. As secreted proteins, the CSFs have signal peptides which are subsequently cleaved from the amino-terminal to yield the mature product. However, certain extended forms of murine Multi-CSF and GM-CSF mRNA encode atypical N-terminal hydrophobic sequences13 and M-CSF has a hydrophobic C-
terminal sequence\(^8\) which suggests that they may function in a membrane-bound form. This could be important in view of the close spatial relationship between haemopoietic progenitor cells and stromal cells in the marrow, and the low, often undetectable, circulating levels of CSF under normal steady-state conditions. Activation of T cells results in the rapid but short-lived secretion of Multi-CSF, GM-CSF and Eo-CSF\(^14\) as well as other lymphokines, and it appears that their production is limited at least partially by post-transcriptional control mediated by an area in the 3'-untranslated region which destabilizes the mRNA.\(^15\) Although it is not clear how CSF production is co-ordinated, it is interesting that the gene encoding murine GM-CSF is closely associated on chromosome 11 with the gene encoding murine Multi-CSF\(^2\) and shares upstream regulatory sequences with it and human GM-CSF.\(^3,18\)

### Actions

The CSFs regulate the production and function of mature haemopoietic cells both \textit{in vitro} and \textit{in vivo}. Multi-CSF acts on stem cells and the progenitors of all non-lymphoid lineages, including mast cells, although its relative activity on each is variable (G. Kanourakis, personal communication); GM-CSF acts on the neutrophil, eosinophil and macrophage lineages, but at higher concentrations may also act on others; G-CSF, M-CSF and Eo-CSF at low concentrations act predominantly on neutrophil, macrophage and eosinophil lineages respectively. In addition to inducing differentiation in normal responsive cells, G-CSF also induces the differentiation and concomitantly reduces the leukaemogenicity of a murine myelomonocytic leukaemia cell line, WEHI-3B(D\(^+\)).\(^19\)

The range of effects which CSFs exhibit on mature cells \textit{in vitro} has been most fully documented for human GM-CSF (Table I). The following sequence of events is suggested after CSF is released at a site of inflammation, for example from an activated T cell. Under the influence of GM-CSF and other chemotactic agents, the responding neutrophil moves to the site of production where its further migration is inhibited. There it becomes primed to degranulate and release superoxide in response to bacterial products such as the chemotactic tripeptide, f-met-leu-phe (FMLP). Meanwhile the expression of complement receptors on the neutrophil's surface is enhanced and micro-organisms opsonized by complement or antibody are phagocytosed or killed extracellularly.

In general, the effects on haemopoiesis of injecting recombinant or synthetic CSFs into animals have corroborated what one would have predicted from the known actions of these molecules \textit{in vitro}. Thus mice injected with either Multi-CSF\(^2\) or GM-CSF\(^4\) exhibited dose-related increases in the size of the spleen, the principal accessory site of haemopoiesis in the mouse, but only the former showed significant
elevations in progenitor cell frequency and mast cell number. Furthermore, Multi-CSF also increased the frequency of stem cells (CFU-S). Intrapерitoneal injection of either CSF resulted not only in increased numbers of peritoneal neutrophils, eosinophils and macrophages, but also in markedly enhanced phagocytic activity. Treatment of mice with up to 200 ng GM-CSF three times a day failed to elicit significant changes in blood neutrophil concentration. However, a marked neutrophilia results from treatment of monkeys with human GM-CSF or G-CSF or mice with human G-CSF. The evidence from these preliminary short-term investigations indicates that the observed effects are reversible on withdrawing the treatment and are not overtly harmful.

Pathogenesis of myeloid leukaemia

Before briefly considering some of the evidence for mechanisms involved in clinical and experimental leukaemia, it should be noted that much excitement has arisen in recent years following the recognition of significant homology between oncogene products and proteins involved in the transmission of growth factor signals. Furthermore, some of these oncogenes and the genes for these proteins have been mapped to areas around 'fragile sites' frequently involved in chromosomal rearrangements in specific tumours. Such observations are now being extended to haemopoietic growth factors and leukaemias.

Normal haemopoietic stem cells and progenitor cells are absolutely dependent on exogenous CSFs and differentiate under their influence to generate more mature cells with progressively restricted growth potential. Leukaemia is therefore the growth of a clone of cells in which self-renewal becomes more likely than differentiation and a high proliferative capacity is thus maintained in the progeny. This may result from a receptor/post-receptor abnormality which dissociates the 'balanced' differentiative signal from the proliferative signal in response to binding of exogenous CSFs. Leukaemic cells might also obtain an advantage over normal cells by producing their own proliferative growth factor (the autocrine hypothesis).

In support of the autocrine mechanism, the generation of leukaemic cells from non-leukaemogenic, factor-dependent cell lines in vitro is associated with the acquisition of factor-independence. More directly, the GM-CSF gene was shown to function as an oncogene when it was introduced into a factor-dependent, non-leukaemogenic cell line (FDC-P1) which consequently secreted the growth factor, became independent of exogenous CSF in vitro, and produced leukaemia in vivo. Although the insertion of the GM-CSF gene was sufficient to render previously immortalized FDC-P1 cells leukaemogenic, it is likely that at least two events are necessary to achieve this in normal cells. Although the vast majority of primary human myeloid leukaemias are absolutely dependent on CSF for their proliferation in vitro, a limited number of examples have recently been reported, in which primary acute myeloblastic leukaemia cells were shown to contain GM-CSF transcripts.

Autocrine mechanisms do not seem to be involved in other experimental systems. The v-fms oncogene product is homologous to a truncated form of the MC-CSF receptor which lacks a portion of the intracellular domain and which might result in signal transmission independently of the growth factor. Insertion of v-fms into an M-CSF (CSF-1) dependent cell line resulted in factor independence and leukaemogenicity. The M-CSF receptor gene is located close to the genes encoding M-CSF and GM-CSF, just distal to the site of chromosomal deletion in the 5q- syndrome, however it is not clear what role this genetic abnormality plays in the development of the refractory anaemia and late leukaemia which characterize the syndrome.

An additional mechanism whereby a leukaemic clone might emerge may relate to observations suggesting that CSF levels may be subnormal in the vicinity of the leukaemic cells. Low levels of CSF are produced by marrow stromal cells from patients with acute myeloid leukaemia and both low numbers of fibroblast colony forming cells and suppression of fibroblast growth by leukaemic cells could contribute to a CSF-deficiency in the microenvironment of the bone marrow. Low levels of CSF can support survival and proliferation of normal stem cells, while higher concentrations are required for suppression of self-renewal potential. In addition, a relative deficiency of a molecule that is primarily a differentiative stimulus (e.g. G-CSF) could occur either if cells lacked a receptor for G-CSF (which does not appear to be the case) or if production by marrow stromal cells was suppressed.

Table I The actions of purified, recombinant GM-CSF on mature human neutrophils in vitro

<table>
<thead>
<tr>
<th>Action</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration</td>
<td>20–23</td>
</tr>
<tr>
<td>Polarization</td>
<td>24, 25</td>
</tr>
<tr>
<td>FMLP-induced degranulation</td>
<td>25</td>
</tr>
<tr>
<td>FMLP-induced O₂ production</td>
<td>22, 25, 26</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>25, 27–29</td>
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<tr>
<td>Iodination</td>
<td>25</td>
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<tr>
<td>Complement receptor expression</td>
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<tr>
<td>Antibody-dependent cytotoxicity</td>
<td>25, 27</td>
</tr>
<tr>
<td>Survival</td>
<td>24, 25</td>
</tr>
</tbody>
</table>
Pathogenesis of infectious disease

Infections of normal hosts by pyogenic bacteria or parasitic helminths are characterized by a selective neutrophilia or eosinophilia respectively. Furthermore, eosinophils from individuals with eosinophilia are functionally activated. Since specific CSFs stimulate both the production and function of mature lineage-restricted granulocytes, it is likely that they may be involved in the generation of the appropriate effector mechanism.

T cells are important in the development of localized granulomata and abscesses as well as systemic eosinophilia. Three eosinophil-active murine CSFs are produced by activated T cells-Multi-CSF, GM-CSF and Eo-CSF. Eosinophil progenitor cell numbers are increased in the bone marrow of mice infected with Mesocystoides corti and this is accompanied by an increase in serum Eo-CSF activity. Eo-CSF is also produced by their spleen cells when stimulated by specific antigen in vitro. Similarly, the T cells of mice infected with Schistosoma japonicum produce a GM-CSF in response to soluble egg antigen, although in this case it could be distinguished from the higher molecular weight CSF present in serum. Monocytes are also important as they secrete at least two products in addition to CSFs which stimulate eosinophils, i.e. eosinophil activating factor and tumour necrosis factor (TNF-α). Intraperitoneal injection of Multi-CSF or GM-CSF into mice resulted in a local increase in eosinophils, and treatment of mice with Multi-CSF or monkeys with an infusion of human GM-CSF stimulated peripheral blood eosinophilia, which supports a role for these factors in this process.

Macrophages activated by lymphokines released from antigen-specific T cells are important in the elimination of intracellular infections by parasites or certain bacteria, particularly Mycobacteria. Interferon-γ is perhaps the most potent of these factors, however CSFs also stimulate several parameters of mature macrophage function, including parasite killing.

The peritoneal macrophages of mice treated with CSF are morphologically activated, show enhanced phagocytosis and may also function more effectively as antigen-presenting cells.

CSF is undetectable in the serum of germ-free mice, is present at low concentrations following bacterial colonization and is elevated during clinical or subclinical infections. Urinary CSF levels are also increased during infections. Bacterial endotoxin induces rapid, 100-1000-fold rises in serum CSF levels within hours of injection and remains one of the most potent stimuli for CSF production. The sources and mechanisms are uncertain but may involve the concomitant release of TNF, which stimulates GM-CSF production by vascular endothelial cells and which mediates the cachexia associated with severe chronic infections. Certainly the reciprocal interactions between different cytokines in vitro are complex: CSFs stimulate monocytes/macrophages to produce interleukin 1, interferon, TNF and other CSFs, while interferon-γ enhances their production of CSF. The actions of these factors may be synergistic or apparently antagonistic since TNF, like GM-CSF, stimulates mature neutrophils and eosinophils but inhibits the growth of some haemopoietic progenitor cells. Thus the outcome is dependent on many variables, some of which may be amenable to therapeutic intervention.

Therapeutic uses of CSFs

The actions of the CSFs in enhancing the production, function and survival of normal haemopoietic cells as well as the differentiation and clonal extinction of leukaemic cells indicate that CSFs soon may become extremely useful prophylactic and therapeutic agents in several areas of clinical medicine. However, some observations from studies in vitro and in vivo predict potential side effects which certainly should be considered as clinical trials begin. For instance, GM-CSF induces neutrophil aggregation in vitro and pulmonary infiltration by neutrophils in vivo although these effects are observed without stimulating their adherence to endothelium in vitro or overtly impairing respiratory function. Direct stimulation of interleukin 1 release rather than potential immunogenicity may have caused fever in monkeys treated with human GM-CSF and release of TNF may mediate the severe shock-like side effects which have already limited the clinical use of this agent itself. Bone marrow-derived osteoclasts are also responsive to CSFs, so assessment of bone mass and turnover probably should not be omitted from any protocol involving their long-term administration. The generation of functionally immature monocytes may provide 'safe targets' in which intracellular parasites such as Leishmania may reside. Finally, a recent report that GM-CSF activates a latent human immunodeficiency virus infection in vitro is of some concern (Science, 236, 1627, 1987). Despite these reservations, early results have been encouraging.

Current treatment modalities for acute leukaemia include chemotherapy and bone marrow transplantations. Administration of the former may be limited by bone marrow suppression which necessitates appropriate supportive therapy, including transfusions. CSFs maintain the survival of granulocytes in vitro and may become routine additives to granulocyte transfusion bags. Furthermore, the administration of G-CSF to monkeys or mice hastened recovery from cyclophosphamide-induced neutropenia and enhanced their resistance to infection (S. Asano, personal communication). The mechanism of this
action is unknown but G-CSF may act indirectly on stem cells through release of a second factor. Similarly, CSFs may be useful in bone marrow transplantation to maintain the cells \textit{ex vivo} and stimulate repopulation when administered \textit{in vivo}, since Multi-CSF stimulates haemopoietic recovery following irradiation.\(^3\)

If leukaemia results because of a failure of a particular clone to undergo differentiative divisions with subsequent loss of self-renewal capacity, then a potential approach to therapy would be to promote differentiation by the administration of an exogenous inducing agent to which the cells remain responsive. The CSFs can induce differentiation in murine and human leukaemic cells, as evidenced by morphological changes, expression of surface antigens and the acquisition of mature functional activity.\(^7,\(^3\) In some situations differentiation induction can be enhanced as a result of an interaction between chemical or cytotoxic agents and CSF.\(^4\)

Differentiation induction by CSF has been studied most extensively with the murine leukaemic cell line WEHI-3B (D\(^+\)). G-CSF can stimulate these cells to differentiate to functionally active, post-mitotic cells with complete loss of self-renewal potential and extinction of the leukaemic clone.\(^1\) Induced differentiation and reduced clonogenicity of the leukaemic cells can be translated into a survival advantage for the animals bearing such a tumour when treated with G-CSF.\(^7\) Cells failing to respond to G-CSF in this manner fail to bind the ligand presumably lack the receptor.\(^8\) Similar studies using a human leukaemic cell line, HL60, have shown that GM-CSF and G-CSF stimulate differentiation with the ultimate extinction of the leukaemic clone.\(^6\)

Primary human myeloid leukaemia cells express receptors for G-CSF whose binding characteristics appear to be indistinguishable from those present on normal cells.\(^77\) Their proliferation \textit{in vitro} is absolutely dependent on CSF and their responsiveness to CSF is generally similar to that of normal cells\(^7\) although hyporesponsive and hyperresponsive populations have been observed.\(^79,\(^80\) Since differentiation of primary myeloid leukaemia cells and leukaemic cell lines induced by CSF \textit{in vitro} is preceded by a wave of proliferation,\(^1,\(^76\) and since unresponsive subsets of leukaemic cells may exist \textit{de novo} or arise as a result of treatment with CSFs their future use in patients should be cautious.

CSF levels are usually elevated in response to infections and it remains to be seen whether any benefit accrues from treating infected but otherwise immunocompetent patients with CSFs. However, their administration to compromised hosts certainly has a logical basis. Apart from their use in secondary immunodeficiency states associated with neoplasia and its treatment, CSFs may also find a role in the prevention and treatment of intercurrent infections in patients with primary immunodeficiency and the acquired immunodeficiency syndrome (AIDS). Studies will soon determine whether neutrophils from children with X-linked chronic granulomatous disease respond to CSF \textit{in vitro}. Although it is unlikely that functions which specifically depend on the deficient cytochrome would be stimulated, CSF administration may nevertheless result in a clinically significant response through other pathways. Haematological improvements were seen in monkeys infected with a simian immunodeficiency virus\(^8\) and phase I trials have already begun in patients suffering from AIDS.

The recent success of the rational approach to developing a cholecystokinin antagonist raises hopes that others may be produced which alter binding of other polypeptides to their receptors.\(^81\) A major advance has been the quantitative chemical synthesis of large polypeptides, including CSFs,\(^82\) which should facilitate structure-function analysis and monoclonal antibody production. If the expected suppression of normal myelopoiesis can be minimised, such antagonists and antibodies may find a use in the treatment of CSF-dependent leukaemias, unless endogenous production allows intracellular ligand-receptor interaction\(^4\) or in the treatment of inflammatory diseases like rheumatoid arthritis in which CSFs may play a role.\(^83\)

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COLONY-STIMULATING FACTORS IN DISEASE

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