

stage where we realize that the introduction of chaos can be quite useful or desirable. Generally, so far, in physical, mechanical and engineering systems the stress has been to avoid nonlinearity so as to be free from any unpredictable motions of dynamical systems. Only now have researchers begun to use the advantages of designing systems to exploit, rather than avoid, nonlinearity and chaos. One dividend for such an outlook is the realization of the possibility of synchronization of chaotic orbits, leading to new vistas in secure signal transmission and thereby harnessing chaos.

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REVIEW ARTICLE

The biology of human granulocyte colony stimulating factor

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Human granulocyte colony stimulating factor (hGCSF) is one of the important haemopoietic growth factors which mediates granulopoiesis and has several potential therapeutic applications. It is a glycoprotein with the number of amino acids 177 and 174 for its two forms which are produced as a result of alternative splicing of precursor mRNA. In the body it is produced by monocytes, fibroblasts and vascular endothelial cells in very minute quantities. In clinical practice hGCSF is used in the patient with agranulocytosis and granulocytopenia, congenital in nature or as a result of bone marrow transplant or myelosuppressive chemotherapy during the treatment of several types of cancers and AIDS.

THE production of mature blood cells from pluripotent stem cells, derived from bone marrow and incapable of unstimulated division, is a complex and intricate process that requires the well-coordinated and concerted action of several protein molecules which are collectively known as hemopoietic growth factors. Of these, colony stimulating factors (CSFs) are a group of proteins which regulate the proliferation and differentiation of the cells of granulocyte and macrophage lineages. In murine and human beings, the presence of four CSFs have been clearly established. Of these, two factors namely, interleukin-3 (Multi-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) are broad range in their activities supporting the growth of granulocyte, macrophage, eosinophil and other hemopoietic pro-

genitor cells. The remaining two CSFs, namely, macrophage colony stimulating factor (M-CSF) and granulocyte colony stimulating factor (G-CSF) are relatively lineage specific mediating the proliferation of macrophages and granulocytes respectively¹. Recently a fifth member of the CSF family has also been recognized as Interleukin-5 that regulates the growth of eosinophil progenitors².

Isolation and characterization of human G-CSF

The earliest evidences of the recognition of granulopoietic activity of certain biological materials coincide with the development of cell culture systems for the cloning and clonal differentiation of certain mouse³ and human⁴ blood cell precursors in which the cells from blood forming tissues were cultured along with a different (feeder) layer of cells supplying the growth factor and separated by semisolid agar medium. The colonies produced in such a system were identified as granulocytic, monocytic or containing both types of cells in different stages of differentiation. Later on, it was shown that an inducer of differentiation of myeloid leukemia cells was also present in the conditioned media of certain normal⁵ and malignant cell lines⁶. The first inducer identified was named as 'mashran gm' from the Hebrew word which means 'to send forth' and the initials for granulocytes and macrophages⁷. Subsequently, attempts were directed by several groups to purify active components that led to the differentiation of myeloid leukemia cell lines and were generally named as MGIs (macrophage, granulocyte inducers) and CSFs. With the application of techniques for the purification of proteins at higher resolution it became increasingly clear that a distinct glycoprotein fraction existed, containing the activity of producing predominantly granulocytic colonies in *in vitro* culture systems and which exhibited exceedingly high ability to differentiate myeloid leukemia cell lines (WEHI-3B). This fraction was termed as Mgl-2G (ref. 8), Differentiation factor (DF)⁹ and finally G-CSF¹⁰ revering to its primary proliferative activity on granulocyte macrophage precursors and murine G-CSF was successfully isolated to homogeneity¹⁰ from medium conditioned by lungs from mice injected with bacterial endotoxin.

The available literature indicates that three research groups were actively engaged in isolation and characterization of the human analogue of murine G-CSF. Nicola and coworkers¹¹ reported for the first time the existence of two molecular species of granulocyte macrophage CSF from human placental conditioned medium. The two fractions identified as GM-CSFa and GM-CSFb, appeared to have similar molecular weight (30,000) and isoelectric point (4.9) and could be clearly

distinguished functionally. The latter form stimulated the formation of colonies in clonal culture systems that reached maximal size and numbers at day 7 of incubation and contained predominantly neutrophilic granulocytes while the other fraction produced colonies composed of several types of cells which attained maximal size and numbers at day 14 of incubation. The same group subsequently identified GM-CSFb as the human analogue of murine G-CSF as it showed differentiation inducing activity on murine myeloid leukemia cell line WEHI-3B⁺.

Efforts to isolate hG-CSF to maximum purity by other groups were more fruitful thanks to the successful establishment of cell lines producing large amounts of CSFs constitutively. From one such human bladder carcinoma cell line 5637, a CSF was purified to near homogeneity using low serum containing conditioned medium. The purified protein was termed as pluripotent CSF and was reported to have molecular weight 18,000 and isoelectric pH 5.5 and supported the growth of human mixed granulocyte, macrophages and early erythroid colonies and induced differentiation of the human promyelocytic leukemia cell line HL-60 and murine myelomonocytic leukemia cell line WEHI-3B (D+). The pluripotent CSF was further purified to near purity (>95%) and was subjected to N-terminal amino acid sequence analysis to deduce oligonucleotide probe to be used for isolating cDNA clones in *E. coli*.¹²

Similarly, from serum-free medium conditioned by another constitutively hG-CSF producing human squamous cell carcinoma line CHU-2, hG-CSF was isolated and characterized in SDS-polyacrylamide gel electrophoresis as a homogeneous identity of greater than 90% purity with molecular weight 19,000 (ref. 13).

The success in obtaining hG-CSF to greater than 90% purity tempted researchers to determine its partial amino acid sequence. By that time the techniques of recombinant DNA technology had developed to the point that by making use of partial amino acid sequence, oligonucleotide probes with deduced sequence could be synthesized and used to identify hG-CSF gene containing clones from the cDNA library prepared from carcinoma cell lines constitutively producing hG-CSF^{14, 15}. The hG-CSF cDNA clones were sequenced and expressed in *E. coli*¹⁴ and monkey COS cells¹⁵, giving rise to recombinant hG-CSF showing activities similar to native protein. While comparing the amino acid sequence of pure hG-CSF protein with that deduced from nucleotide sequence of hG-CSF cDNA, it was found that there was a definite difference at one place between them. This led to the search for cDNA coding for another form of hG-CSF by hybridizing with a different oligonucleotide probe designed to contain possible nucleotide sequence for the other form¹⁵. Thereafter, the shorter version of hG-CSF which has a deletion of 3 amino acids at positions 36–38 was also isolated followed by the expression of its cDNA in

MetThrProLeuGlyProAlaSerSerLeuProGlnSerPheLeuLeuLysCys
 ATGTCCCCCTGGGCCCTGCCAGCTCCCTGCCCCAGAGCTTCCTGCTCAAGTGC-

LeuGluGlnValArgLysIleGlnGlyAspGlyAlaAlaLeuGlnGluLysLeu
 TTAGAGCAAGTGAGGAAGATCCAGGGCGATGGCGCAGCGCTCCAGGAGAAGCTG-

ValSerGluCysAlaTheTyrLysLeuCysHisProGluGluLeuValLeuLeu
GTGAGTGAGTGTGCCACCTACAAGCTGTGCCACCCGAGGAGCTGGTGCTGCTC-

GlyHisSerLeuGlyIleProTrpAlaProLeuSerSerCysProSerGlnAla
 GGACACTCTCTGGGCATCCCCTGGGCTCCCCTGAGCAGCTGCCCCAGCCAGGCC-

LeuGlnLeuAlaGlyCysLeuSerGlnLeuHisSerGlyLeuPheLeuTyrGln
 CTGCAGCTGGCAGGCTGCTTGAGCCAACTCCATAGCGGCCTTTTCGTCTACCAAG-

GlyLeuLeuGlnAlaLeuGluGlyIleSerProGluLeuGlyProThrLeuAsp
 GGGCTCCTGCAGGCCCTGGAAGGGATCTCCCCGAGTTGGGTCCACCTTGGAC-

ThrLeuGlnLeuAspValAlaAspPheAlaThrThrIleTrpGlnGlnMetGlu
 ACACTGCAGCTGGACGTCGCCGACTTTGCCACCACCATCTGGCAGCAGATGGAA-

GluLeuGlyMetAlaProAlaLeuGlnProThrGlnGlyAlaMetProAlaPhe
 GAACTGGGAATGGCCCCCTGCCCTGCAGCCCCACCCAGGGTGCCATGCCGGCCTTC

AlaSerAlaPheGlnArgArgAlaGlyGlyValLeuValAlaSerHisLeuGln
 GCCTCTGCTTTCCAGCGCCGGGCAGGAGGGGTCTGGTTGCCTCCCATCTGCAG-

SerPheLeuGlyValSerTyrArgValLeuArgHisLeuAlaGlnProEnd
 AGCTTCCTGGAGGTGTCGTACCGCTTCTACGCCACCTTGCCCAGCCCTGA

Figure 1. The nucleotide and deduced amino-acid sequence of the cDNA encoding mature hGCSFa with Thr at position 1. The underlined portion is spliced out in hGCSFb form.

monkey COS cells¹⁵ (see Figure 1). Subsequently, the artificial genes coding for the two forms of the hG-CSF were synthesized by chemico enzymatic method¹⁶ with the aim of their expression in heterologous hosts¹⁷.

Production of HG-CSF

Major sources of G-CSF in the body are monocytes/macrophages, vascular endothelial cells and fibroblasts which produce G-CSF only on some kind of stimulation. Intriguing is the fact that although granulopoiesis is a continuous process in the body, normal cells producing G-CSF at protein or mRNA level constitutively, have not been recorded so far¹⁸. Some carcinoma cells such as squamous carcinoma line CHU-2, bladder carcinoma line 5637, however, produce G-CSF and other CSFs constitutively in large amounts. Bacterial lipopolysaccharide is known to be a potent inducer of G-CSF which stimulates monocytes directly and endothelial cells and fibroblasts indirectly to produce G-CSF both at mRNA and protein levels¹⁹. In addition, a variety of mitogenic stimuli such as *con* A, PMA (Phorbol 12-myristate 13-acetate) etc. also induce monocytes to produce G-CSF but require the cooperative action of T-lymphocytes¹⁹ probably through interferon²⁰. T-cell derived cytokines such as GM-CSF and IL-3 have been shown to activate monocytes,

independently as well as synergistically to transcribe G-CSF mRNA as well as to translate protein *in vitro*²¹. Interleukin 1 modulates the levels of G-CSF and GM-CSF in vascular endothelial cells²². Tumor necrosis factor (TNF) α but not β stimulates the production of G-CSF in fibroblasts²³. In endothelial cells, however, TNF α does not exert stimulatory effect²⁴. According to a recent report, polymorphonuclear leukocytes have also been shown to secrete G-CSF upon induction by GM-CSF indicating the autoregulatory control mechanisms for its production²⁵.

Taking all these facts together, a scheme for the modulation of G-CSF levels in the body and for granulopoiesis can be drawn up. Invasion of bacteria as a result of tissue injury, stimulates macrophages at the inflammatory site to release TNF and IL-1, which in turn, induce local mesenchymal cells to secrete G-CSF and other factors. These factors activate granulocytes and other phagocytic cells to migrate at inflammatory foci and ensure their retention resulting in phagocytosis and killing of invading bacteria.

Molecular biological properties of HG-CSF

Human granulocyte colony stimulating factor, a monomer glycoprotein with molecular weight about 19,600, exists in two forms as has been detected at least in cell line CHU-2, constitutively producing hG-CSF. The two forms of hG-CSF are first synthesized as precursor molecules containing 207 and 204 amino acids of which 30 amino acids at the N-terminal end are the signal sequences allowing secretion of hG-CSF molecules. Thus the mature forms of hG-CSF hereafter termed as hG-CSFa and hG-CSFb consist of 177 and 174 amino acids respectively. More than 80% of the G-CSF molecules produced in CHU-2 cells belong to the shorter i.e. hG-CSFb version²⁶. The theoretically deduced amino acid sequence, later confirmed by experimental analyses with recombinant hG-CSF, suggests the presence of 5 cysteines, 4 of which take part in forming two disulphide bridges located at positions 36, 42 and 67, 77. The cysteine residue placed at position 17 is free and is inaccessible to chemical modification unless the molecule is unfolded²⁷. Circular dichroism spectra of bacterially produced rhG-CSF has revealed that hG-CSF is a globular protein molecule with probable 69% alpha helical structure, 4% beta sheet and 5% beta turn structure. The hydrophilicity profile of the same molecule has indicated it to be strongly hydrophobic²⁷.

It is interesting to note that contrary to all other CSFs which are N-glycosylated, G-CSF is unique in having O-glycosylated sugar chain, linked to threonine at positions 136 and 133 in hG-CSFa and hG-CSFb molecules respectively. The structure of O-linked sugar chain has also been elucidated to be neuraminic acid

$\alpha(2-6)[\text{galactoso } \beta(1-3)] \text{ N-acetyl galactosamine-R}$; where R is thr¹². It has also been shown that glycosylation is not essential for the functional activities of a hG-CSF molecule. However, available evidences do indicate that sugar chain protects the molecule against polymerization and denaturation since the deglycosylated molecules have been found to be more heat sensitive²⁸. A novel derivative of hG-CSF with N-linked sugar chain has also been experimentally constructed by introducing N glycosylation site (Asp-x-Thr/Ser) in the molecule by site-directed mutagenesis. The N-glycosylated form appeared to be more thermostable and protease resistant with identical functional specific activity²⁹.

The amino acid sequence of human G-CSF shares considerable (72%) homology with mouse G-CSF which can be correlated to the lack of species specificity between the two. Most of the divergence in amino acid sequences is restricted to N terminal region suggesting the conservation of C-terminal region which is necessary for the functional activity²⁶. In mouse however, only one kind of G-CSF molecule has been recognized that corresponds to the shorter version of hG-CSF molecule.

In spite of showing some of the functional activities similar to other human CSFs, specially to GM-CSF, hG-CSF shows no apparent homology with IL-3, M-CSF, GM-CSF or IL-5 (ref. 14), instead, it has a significant homology with interleukin-6 (IL-6, also known as B-cell stimulating factor or BSF2) which stimulates the proliferation and differentiation of B lymphocytes³⁰. The homologous region between the two CSFs spans from amino acid position 20 to 93 including the two disulphide bridges indicating possible similarities in their tertiary structures as well.

Several muteins of hG-CSF have been prepared and it has been noted that the N-terminal region is not essential for its functional activity as hG-CSF showed biological activity without at least 11 N-terminal amino acids. Interestingly, replacement of N-terminal sequences by certain other sequences resulted in enhanced specific activity of hG-CSF³¹ and substitution of cysteine at position 17 with alanine increased thermostability³². Recombinant hG-CSF expressed in *E. coli* has been crystallized³³ but the data on conformation of the molecule are not yet available.

Both forms of hG-CSF are encoded by a single chromosomal gene that is located on chromosome 17 at bands q11.121 (ref. 34). This region is proximal to the breakpoint of a reciprocal translocation involving chromosome 15 and 17, a characteristic feature of acute promyelocytic leukemia {t(15:17) (q22; q11.2)}, and it has been shown that G-CSF gene is not rearranged during this translocation³⁵. It is noteworthy that the genes for other human CSFs (IL-3, GM-CSF and M-CSF) are clustered on chromosome 5 at 'CSF' region,

and that the gene for mouse G-CSF showing significant homology to that of human G-CSF in coding regions as well as regulatory regions, is situated on chromosome 11 along with the genes for other CSFs.

The structural gene for hG-CSF, as has been characterized by nucleotide sequence analysis, is about 2.5 kb long and consists of 5 exons interrupted by 4 introns. The exons 1, 3, 4 and 5 are similarly represented in mRNAs for the two forms of hG-CSF but exon 2 is differently utilized. The exon 2 is flanked at its 3'-end with two donor splice sites in series arranged 9 nucleotides apart and the alternative use of these splice donor sites by 5'-end of exon 3 generates two different mRNAs which differ only by 9 nucleotides and in turn are responsible for the production of two molecules, hG-CSFa and hG-CSFb, having a difference of 3 amino acids¹⁵. It will be relevant to mention here that the generation of two forms of hG-CSF, as a result of alternative splicing, has only been recorded in squamous cell carcinoma line CHU-2. It is not yet known which of the two forms, or both, are actually produced in the body. Another interesting feature that has been observed is the preferential splicing pattern of G-CSF mRNA in different cells, e.g. more than 80% of G-CSF mRNA molecules produced in CHU-2 cells are for the G-CSFb form whereas monkey COS cells introduced with cloned G-CSF chromosomal gene produce largely G-CSFa mRNA¹⁵. The significance and mechanism of this tissue specific splicing remains to be explained.

Thus far, the mechanism of the regulation of hG-CSF gene expression is an enigma. However, it has been established that the levels of G-CSF mRNA in human blood monocytes and mesenchymal cells are modulated by transcriptional and posttranscriptional control mechanisms. The recent attempts that have been made to understand the transcriptional control mechanisms have envisaged the presence of at least 3 G-CSF promoter elements (GPE 1, 2 and 3) in cells producing G-CSF constitutively as well as in cells producing it on lipopolysaccharide stimulation³⁶. The regulatory region of human G-CSF gene spans about 300 bp upstream of the initiation codon and shows about 80% homology with that of murine gene³⁷. The GPE 1 region is 42 nucleotides long and consists of a 'CSF box' at the distal region, a sequence GAGATTCCCC that is highly conserved in the regulatory regions of GM-CSF and IL-3 genes³⁷. This sequence is related to the NF-kB element (GGGGGATTTC), that functions as LPS inducible enhancer for Igk gene in pre B lymphocytes³⁶. But contrary to expectation, the proximal region of GPE 1 and not CSF box has been found to mediate cell specific LPS induced expression of hG-CSF gene in macrophages³⁶. Another regulatory element GPE 2 is an octamer sequence (ATTTGCAT), similar to OTF binding site present in promoters of immunoglobulin gene. GPE 3 region has been identified as about 40

nucleotides long. The functions of GPE 2 and GPE 3 regions await further elucidation.

The presence of several copies of ATTTA motif, which is implicated in the instability of mRNA, in hG-CSF mRNA at 3'-end has also been established suggesting the role of posttranslational control mechanisms occurring in monocytes on LPS stimulation³⁸ as well as in mesenchymal cells on induction by TNF α and cycloheximide³⁹.

The receptors for hG-CSF (hG-CSF-R) have been found on human bone marrow cells of myeloid lineage, mature neutrophils⁴⁰, acute myeloid leukemia cells¹², and carcinoma cell lines of hemopoietic (HL-60, KG-1) as well as of nonhemopoietic origin⁴¹. The number of receptor molecules varies from 300 to about 1000 per cell. They bind hG-CSF molecules with K_d of 250 pM. The structure of the receptor molecule has also been unravelled and its cDNA cloned. The GCSF-R belongs to the hemopoietic growth factor receptor superfamily (HRS)⁴² and consists of two types of protein molecules with molecular weight of about 150,000 and number of amino acids 812 and 759 (ref. 43). There are 4 structural motifs in extracellular regions common to both types of receptors which include a set of 4 cysteines, a juxtamembrane Trp-ser-x-trp-ser motif, an amino-terminal region belonging to immunoglobulin superfamily and a threefold repeated fibronectin type III domain. The cytoplasmic region is different in type I and type II receptor molecules with 87 and 34 amino acid residues respectively. The presence of Ig like and FN type III modules in G-CSF-Rs suggests that they might have been derived from neural cell adhesion molecules and may function for some recognition and adhesion functions, in addition to binding of G-CSF molecules⁴³.

hG-CSF and granulopoiesis

Fairly sufficient data are now available which prove that G-CSF plays an important role in the production of mature granulocytes as a part of the normal process of hematopoiesis as well as in the situations of increased demands such as acute bacterial infections, myelo-suppressive chemotherapy and exposure to radiations. The process of the formation of end cells in the lineage i.e. functional neutrophilic granulocytes from multipotential stem cells takes place through several stages each of which involves acquiring of new morphological, functional and membrane marker properties⁴⁴. Irreversible commitment to a particular lineage of the progeny cells from stem cell population probably requires a specific 'microenvironmental niche' provided by stromal cells¹. A recently defined stromal cell derived growth factor (SCF), a product of steel gene, has been postulated to play some role in the process⁴⁵. The committed progenitor cells which

develop into neutrophils are bipotential as they can also give rise to the cells of macrophage lineage in certain conditions⁴⁴.

hG-CSF, native as well as recombinant, when administered into healthy human⁴⁵, rodents⁴⁶, dogs⁴⁷, and primates⁴⁸ in doses as low as 0.3 to 300 mg⁻¹ kg⁻¹ day⁻¹, significantly increases the number of circulating granulocytes and correspondingly the absolute leukocyte numbers within 12 hours after a single injection. Thus, the effect of hG-CSF is rapid, potent and highly specific i.e. restricted to the increment in cells of granulocytic lineage only, with no effect on lymphocyte, monocyte or eosinophil lineages. The number of progenitor cells in the granulocyte lineage is also increased in dose-dependent manner. Similar results have been reported on administration of rhG-CSF in chemotherapy induced neutropenic rodents⁴⁹, monkeys⁵⁰ and human patients⁵¹ and in total body irradiated dogs⁴⁷. The half-life ($t_{1/2}$) of elimination of G-CSF in human beings has been recorded as 1.3–4.2 hours⁵². The extended neutrophilia on hG-CSF administration reflects the replenishment of progenitor cells from stem cells. Neutrophil counts return to preinoculation stages within 2–3 days after discontinuing the G-CSF administration.

hG-CSF and end cell functions

hG-CSF is not only a regulatory molecule which exerts its proliferative action on progenitor cells of granulocytic lineage but it also profoundly potentiates the functional activities of post-mitotic end cells in the series. It prolongs the survival of mature granulocytes, enhances neutrophil mediated antibody dependent cellular cytotoxicity (ADCC), primes the neutrophilic cells for the production of superoxide anion in response to bacterial chemotactic peptide fMet Leu Phe and increases the phagocytic capacity of neutrophils.

Since the time of initial characterization of hG-CSF it has been known that it has two sites of actions on the cells of granulocytic series namely, at progenitor cell stage and at the post-mitotic stage. The earlier experiments carried out with purified mouse G-CSF on human neutrophils revealed that it enhanced ADCC by neutrophils particularly at low antibody concentrations⁵³. Comparable results were recorded with human G-CSF on tumor target cells. GM-CSF and G-CSF showed additive effect in enhancing ADCC⁵⁴. In the last few years, significant progress has been achieved in understanding the mechanism of enhancement of cytotoxic activity of neutrophils using purified recombinant hG-CSF. It appears that hG-CSF primes granulocytes for the release of O³⁻ and membrane depolarization by chemotactic peptide fMet Leu Phe and wheat germ agglutinin, and not by Ca⁺⁺ ionophore ionomycin and PMA⁵⁵. The reaction occurs in a manner that does not require any new protein

synthesis. The recent experiments indicate that during the priming of granulocytes, there is no effect on resting transmembrane electrical potential and intracellular concentration of free Ca^{++} ions, and there is no induction of translocation of protein kinase C as well⁵⁶. But hG-CSF directly causes release of arachidonic acid from plasma membrane phospholipids representing the receptor-mediated activation of membrane phospholipases⁵⁷. It remains to be fully explained how the release of arachidonic acid is related to the increased cytotoxic activities of neutrophils. However, it has been proposed that a derivative of arachidonic acid metabolite via lipoxygenase pathway, leukotriene B4 may be active in this capacity⁵⁸. The triggering of ADCC by hG-CSF is especially significant against human immunodeficiency virus (HIV) infected cells since neutrophils are apparently not infected with the virus, whereas the other cells are, and this reaction may be a prospective line to develop for AIDS therapy⁵⁹.

HG-CSF also causes an increase in the expression of C3bi receptors which are adhesion specific molecules and this reaction results in enhanced adherence of granulocytes in *in vitro* experiments⁵⁶. It partly explains the state of transient neutropenia observed following the administration of hG-CSF in animals or human beings which is probably a result of the aggregation of neutrophils followed by adhesion to vessel wall.

On inoculation of rhG-CSF in human patients the granulocyte-specific enzymes such as lysozymes and elastase have been observed to increase which suggest the augmentation of the functions of mature neutrophils by hG-CSF⁴⁵.

Effects of HG-CSF on myeloid leukemic cells

Myeloid leukemia is a disease condition characterized by increased number of the cells of granulocyte macrophage lineage in all stages of maturation and relative accumulation of immature granulocyte-monocyte blast cells in circulation with chronic or acute courses. The *in vitro* growth of myeloid leukemic cells is evidenced by elevated frequency of colony forming cells, i.e. their increased clonogenicity¹. The initial findings with murine G-CSF, which shows several properties including receptor binding similar to human G-CSF on cultured myeloma cell lines, were highly encouraging as it terminally differentiated murine myelomonocytic leukemic cell line WEHI-3B(D)⁺ into granulocytes and monocytes⁶⁰. The differentiation of this murine leukemic cell line was found to be irreversible with suppression of the capacity of self-generation evidenced by loss of clonogenicity and appearance of surface antigens and membrane markers of mature cells. Subsequently, it was shown that human cells from leukemic patients bound ¹²⁵I labelled murine G-CSF and the binding was competitively inhibited by

unlabelled hG-CSF⁶¹. Similarly, purified murine G-CSF and semipurified human G-CSF appeared to primarily accord differentiative action on human HL-60 (promyelocytic stage of differentiation) cell line⁶² but in some instances proliferative action was found to be more pronounced⁶³. With the availability of recombinant human G-CSF, more detailed analysis of the effects of hG-CSF on myeloid leukemic cells could be conducted. RhG-CSF was found to bind to human myelomonocytic leukemic cells and to induce granulocytic and monocytic differentiation of bone marrow cells from patients with acute promyelocytic leukemia and acute myeloblastic leukemia¹². Further reports on the action of hG-CSF on myeloid leukemic cells show heterogeneity⁶⁴. It is probably due to the contradictory role of G-CSF on hemopoietic progenitor cells of inducing proliferation i.e. self-generation and promoting differentiation into nondividing mature cells i.e. suppression of self generation. Similar diversity in the response of hG-CSF has been observed on myeloid leukemic cell lines and leukemic blasts cells from patients with leukemia. Certain leukemias differentially express or lack receptors for hG-CSF¹² but it is not a strict indication of the proliferative response of hG-CSF or clonogenicity⁶³. Therefore, certain cell lines e.g. WEHI-3B(D⁺), HL-60, U937 (the monoblast stage of differentiation) appear to undergo partial differentiation into mature cell stages^{65, 66} and on the other hand the same and some other cell lines (KG-1 and AML blast cells) have been found by other workers to exhibit proliferative response indicating that hG-CSF acts as a growth factor⁶⁷. There are now evidences accumulating which indicate that the proliferative and differentiative actions of hG-CSF can be dissociated. For example, γ -interferon if used in conjunction with hG-CSF, markedly enhances the differentiative action of hG-CSF at least on HL-60 cell lines suppressing its proliferative action⁶⁸. Similarly a combination of hG-CSF and retinoic acid has been found more active in inducing granulocytic maturation than the either factor alone⁶⁹. Other combinations of hG-CSF and GM-CSF, IL-3 and leukemia inhibitory factor (LIF) also appear to be more effective in enhancing differentiation and maturation in HL60 and U-937 cell lines as indicated by suppression of clonogenicity and morphological changes⁷⁰.

Clinical applications of HG-CSF

Granulocytes serve as an important means of defence in the body. G-CSF, being capable of elevating the levels of circulating granulocytes, appears to be a very useful potential chemotherapeutic agent to boost up the defence mechanisms of the body in several disease conditions. Phase I, II and III clinical trials have already been undertaken and within the last two years, several reports of successful application of hG-CSF in many

patients suffering from variety of disease conditions have accumulated.

Early phase I/II studies of rhG-CSF combined with intensive chemotherapy in malignant conditions have been published by several groups which include patients with small lung cancer⁷¹, urothelial cancer⁷² and pulmonary cancer⁷³ with intravenous or subcutaneous mode of administration. In trial studies the effects of rhG-CSF have also been measured in conditions of myelosuppression associated with intensive chemotherapy with⁵² or without autologous bone marrow transplantation^{71, 74}. These studies lead to the conclusions that intravenous or subcutaneous administration of rhG-CSF causes an initial immediate transient fall in neutrophil count which is followed by a tremendous increase in dose-dependent manner and the duration of neutropenia is shortened significantly. The number of other cells practically remains unaffected³⁰. The increase in the number of circulating neutrophils is collinear with the increased serum levels of alkaline phosphatases, lactate dehydrogenase, uric acid, elastase and lysozymes. The adverse effects which have been noticed are mild and include chest and/or low back pain⁷³ and slight bone pain⁷¹. No serum antibody has been reported to appear against hG-CSF⁷⁵ even after 10 months of treatment.

Administration of rhG-CSF in patients with cyclic neutropenia, who had a history of recurrent bacterial infections, increased the mean neutrophil counts from $717 \pm 171 \text{ ml}^{-1}$ to $9814 \pm 2198 \text{ ml}^{-1}$ and the cycle-length was decreased from 21 to 14 days. During the 40 months of long therapy, the frequency of bacterial infections was significantly reduced⁷⁶. Similar increase in circulating functional neutrophil count was observed in patients of chronic idiopathic neutropenia^{75, 77}. The pattern of hemopoietic response in patients with congenital agranulocytosis or Kostmann's syndrome, characterized by severe absolute neutropenia however, was different. These patients required eight to nine days of treatment before the neutrophil counts could be elevated to more than $1000 \text{ cells ml}^{-1}$. Clinical studies in patients with myelodysplastic syndromes indicated 5 to 40-fold increase in neutrophil counts over 8 weeks of treatment with hG-CSF⁷⁸.

Recently a plethora of reports has appeared enlisting the application of rhG-CSF in several other disease conditions and it is beyond the scope of this review to mention them all. Some of the important reports include its use in aplastic anemia⁷⁹, malignant lymphoma⁸⁰ and allogeneic bone marrow transplantation⁸¹. Since hG-CSF increases ADCC against HIV infected cells⁵⁹, it has been tried in patients with AIDS⁸² and AIDS-related complex⁸³ in combination with azidothymidine and other myelosuppressive antiviral agents. This has resulted in increased neutrophilic counts which is beneficial in preventing and treating secondary infections.

It has been mentioned in a previous section that hG-CSF at times exerts proliferative response in some leukemic cell lines which warrants caution in its application in leukemic conditions. However, administration of hG-CSF in leukemic patients did not seem to have proliferative response⁸⁴. Nevertheless, several further clinical trials need to be done before drawing any conclusions regarding the role of hG-CSF in leukemia therapy.

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