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G-CSF and GM-CSF in Neutropenia

Hrishikesh M. Mehta,* Michael Malandra, † and Seth J. Corey**

G-CSF and GM-CSF are used widely to promote the production of granulocytes or APCs. The U.S. Food and Drug Administration approved G-CSF (filgrastim) for the treatment of congenital and acquired neutropenias and for mobilization of peripheral hematopoietic progenitor cells for stem cell transplantation. A polyethylene glycol–modified form of G-CSF is approved for the treatment of neutropenias. Clinically significant neutropenia, rendering an individual immunocompromised, occurs when their number is <1500/μl. Current guidelines recommend their use when the risk for febrile neutropenia is >20%. GM-CSF (sargramostim) is approved for neutropenia associated with stem cell transplantation. Because of its promotion of APC function, GM-CSF is being evaluated as an immunostimulatory adjuvant in a number of clinical trials. More than 20 million persons have benefited worldwide, and >$5 billion in sales occur annually in the United States. The Journal of Immunology, 2015, 195: 1341–1349.

Few physician-scientists have made as great an impact on our understanding of hematology or improved the lives of patients, estimated at >20 million (1), with blood and cancer disorders as Don Metcalf, who died in December of 2014. Laboring at the Walter and Eliza Hall Institute in Melbourne, Australia throughout his 50-year career, Metcalf used semisolid medium and cell culture supernatants to discover hematopoietic progenitor cells (e.g., granulocyte/macrophage colonies) and their growth factors (e.g., G-CSF and GM-CSF). Increased purification of these and related growth factors, sometimes from hundreds of mice injected with endotoxin, led to the molecular characterization and cloning of G-CSF, GM-CSF, M-CSF, stem cell factor, and IL-3 in the 1980s (2).

Hematopoiesis is a highly proliferative (∼10¹⁰ cells/d) dynamic process driven by multiple hematopoietic growth factors/cytokines (Fig. 1A). The hematopoietic growth factors are multifunctional and are critical for proliferation, survival, and differentiation of hematopoietic stem, progenitor, and precursor cells to a terminally differentiated, functional cell type. Colony-forming assays identified the ability of first crude supernatants, and then highly purified cytokines, to drive multi-lineage and single-lineage differentiation. After coculturing for 7–14 d, colonies from mononuclear cells obtained from the mouse spleen or bone marrow were measured in semisolid medium. Based on the characteristics of cells within a single colony, the lineage(s) governed by the cytokine was determined. Granulocytes make up the majority of WBCs in human circulation and play an integral role in innate and adaptive immunity. In granulopoiesis, their production is mediated by a number of growth factors, especially G-CSF and GM-CSF (3, 4). Due to asymmetric division, some daughter cells of the hematopoietic stem cell (HSC) remain as HSCs, preventing the depletion of the stem cell pool (5). Multiparameter immunophenotyping has transformed our ability to identify different cell types in hematopoiesis. Murine HSCs are characterized as lin− ccr1−c-kit+, and human HSCs display CD34 in the absence of lineage markers. The differentiation pathway from HSCs to granulocytes is dependent on G-CSF and, less so, on GM-CSF. The HSC gives rise to a common myeloid progenitor and a common lymphoid progenitor cell (6). The common myeloid progenitor cells differentiate into myeloblasts, erythrocytes, and megakaryocytes via at least two intermediates: the granulocyte/monocyte progenitor cell and the erythrocyte/megakaryocyte progenitor cell. In the granulocytic series, myeloblasts (15–20 μm) are the first recognizable cells by their scant cytoplasm, absence of granules, and fine nucleus with nucleioli in the bone marrow clearly committed to differentiation to granulocytes. Myeloblasts differentiate into promyelocytes, which are larger (20 μm) and begin to possess granules (Fig. 1B). Promyelocytes give rise to neutrophilic, basophilic, and eosinophilic precursor cells. Cell division continues through the promyelocyte stage. Fine specific granules containing inflammatory-related proteins appear during myelocyte maturation. For neutrophils, their size and nuclei become increasingly more condensed as the cells mature through myelocyte, metamyelocyte, band, and the terminally differentiated neutrophil (polymorphonuclear and ~15 μm). During episodes of stress, such as infection, band cells can...
be found in the peripheral blood and are used as a measure of inflammation. The above process is complex, dynamic, and orchestrated by multiple cytokines and their receptors, most notably G-CSF and GM-CSF.

Following Ag stimulation or activation by cytokines, such as IL-1, IL-6, and TNF-α, macrophages, T cells, endothelial cells, and fibroblasts produce and secrete G-CSF and GM-CSF. Of unknown significance, a variety of tumor cells also produce these paracrine growth factors. Glycoproteins with a molecular mass $\sim 23$ kDa, G-CSF and GM-CSF, are now produced through recombinant technology in either *Escherichia coli* or yeast. G-CSF induces the appearance of colonies containing only granulocytes, whereas GM-CSF gives colonies containing both granulocytes and macrophages. Generation of G-CSF (*Gf3*)- and G-CSFR (*Gf3r*)-knockout mice confirmed that G-CSF critically drives granulopoiesis (7). The cognate receptor for G-CSF is a single-transmembrane receptor that homodimerizes upon G-CSF binding. Unlike G-CSF, GM-CSF functions via a two-receptor system involving a specific $\alpha$-chain and a common $\beta$-chain shared by IL-3 and IL-5 (8). However, GM-CSF–knockout mice did not display a perturbation in hematopoiesis (9, 10). Both G-CSF and GM-CSF signal through pathways involving JAK/STAT, SRC family kinases, PI3K/AKT, and Ras/ERK1/2. The receptor complexes are characterized by high-affinity (apparent $K_D \sim 100–500$ pM) and low-density (50–1000 copies/cell). Interestingly, human
G-CSF is functionally active on murine myeloid cells, but human GM-CSF is not. The signaling specificity likely involves nuances in the proximal postreceptor phosphoprotein networks and the distal gene regulatory networks. The molecular pathways and their cross-interactions in determining lineage specificity are critical to the development of more specific therapies. Cloning of human GM-CSF and its expression in bacterial and eukaryotic cells were achieved in 1985 at the Genetics Institute (11), and cloning of G-CSF and its expression in E. coli was achieved a year later at Angen (12). Commercialized by these biotechnology start-ups, G-CSF and GM-CSF revolutionized the treatment of patients with congenital or acquired neutropenias and those undergoing stem cell transplantation. Sideline from the treatment of neutropenias by its toxicity profile, GM-CSF is now undergoing a renaissance as an immunomodulatory agent.

G-CSF is approved by the U.S. Food and Drug Administration (FDA) for use to decrease the incidence of infection in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs associated with a significant incidence of severe neutropenia with fever; reduce the time to neutrophil recovery and the duration of fever following induction or consolidation chemotherapy treatment of patients with acute myeloid leukemia (AML); reduce the duration of neutropenia and febrile neutropenia in patients with nonmyeloid malignancies undergoing myeloablative chemotherapy followed by stem cell transplantation; mobilize hematopoietic progenitor cells into peripheral blood for collection and use in allogeneic or autologous stem cell transplantation in whom an HLA-matched donor is not available; and reduce the incidence and duration of complications of severe neutropenia in symptomatic patients with congenital neutropenia, cyclic neutropenia, or idiopathic neutropenia. Forms of G-CSF available worldwide include filgrastim, pegfilgrastim, and lenograstim.

GM-CSF is approved by the FDA to accelerate myeloid recovery in patients with non-Hodgkin’s lymphoma (NHL), acute lymphoblastic leukemia, and Hodgkin’s disease undergoing autologous stem cell transplantation; following induction chemotherapy in older adult patients with AML to shorten time to neutrophil recovery and reduce the incidence of life-threatening infections; to accelerate myeloid recovery in patients undergoing allogeneic stem cell transplantation from HLA-matched related donors; for patients who have undergone allogeneic or autologous stem cell transplantation in whom engraftment is delayed or failed; and to mobilize hematopoietic progenitor cells into peripheral blood for collection by leukapheresis. Forms of GM-CSF available worldwide are sargramostim and molgramostim.

The recommended dosage for G-CSF is 5 μg/kg/d and for GM-CSF is 250 μg/m²/d. Both drugs may be given s.c. or i.v., although randomized clinical trials demonstrate greater efficacy (i.e., decreased duration of neutropenia) without a difference in toxicity for the s.c. route (13). For chemotherapy-induced neutropenia, G-CSF is administered until there are >1000 neutrophils/μL. For congenital neutropenias, the goal is to maintain neutrophil counts ~750/μL. G-CSF is well tolerated. Transient fever and bone pain are more commonly observed in those receiving GM-CSF. Pleural and/or pericardial effusions can also occur in those receiving GM-CSF. Long-term side effects of G-CSF administration, such as osteopenia, are being monitored in patients with severe congenital neutropenia (SCN). One concern is that G-CSF may accelerate the transformation of SCN to myelodysplastic syndromes (MDSs) or AML, associated with acquired mutations in G-CSFR.

**G-CSF and GM-CSF signaling pathways and functional consequences**

The receptors for both GM-CSF and G-CSF belong to the hematopoietin/cytokine receptor superfamily. G-CSFR acts as a homodimer, whereas GM-CSFR is a heterodimer with a shared β-chain with the IL-3R and IL-5R complexes. GM-CSF is expressed primarily on neutrophils and bone marrow precursor cells, which undergo proliferation and eventually differentiate into mature granulocytes. G-CSF binds to G-CSFR, resulting in its dimerization, with a stoichiometry of 2:2 and with a high affinity (Kₐ = 500 pM) (14, 15). Among the activated downstream signal-transduction pathways are JAK/STAT, Src kinases, such as Lyn, Ras/ERK, and PI3K (16). The cytoplasmic domain of G-CSFR possesses four tyrosine residues (Y704, Y729, Y744, Y764) serving as phospho-acceptor sites (17, 18). Src homology 2–containing proteins STAT5 and STAT3 bind to Y704 and Gab2 to Y764. Grb2 couples to both Gab2 and to SOS, permitting signaling diversification, such as Ras/ERK, PI3K/Akt, and Shp2 (19, 20). An alternatively spliced isoform of G-CSFR elicits activation of a JAK/SHP2 pathway (15). The precise physiological roles of protein kinases and their downstream events in G-CSF–induced signaling remain unclear, although some clues are beginning to emerge (21, 22).

GM-CSF binds to the α-chain of the GM-CSFR with a low affinity (Kₐ = 0.2–100 nM), but a higher affinity (Kₐ = 100 pM) occurs in the presence of both subunits. GM-CSF signaling involves the formation of dodecameric supercomplex that is required for JAK activation (23). In addition to the JAK/STAT pathway, GM-CSF activates the ERK1/2, PI3K/Akt, and IkB/NF-κB pathways. Although the α-chain is primarily considered a ligand-recognition unit, it interacts with Lyn, resulting in JAK-independent Akt activation of the survival pathway (24). Thus, differences in receptor expression patterns and known and unknown nuances in signaling pathway circuits account for the functional differences between G-CSF and GM-CSF.

G-CSF and GM-CSF are pleiotropic growth factors, with overlapping functions. GM-CSF also shares properties with M-CSF on monocyte function (25). Both GM-CSF and G-CSF increase chemotaxis and migration of neutrophils, but response kinetics may differ. GM-CSF may be considered to be more proinflammatory than G-CSF. GM-CSF increases cytotoxic killing of Candida albicans, surface expression of Fc- and complement-mediated cell-binding (FcγR1, CR-1, CR-3), and adhesion receptor (14). Yet, both cytokines promote neutrophil phagocytosis (26). More extensive reviews on G-CSF and GM-CSF function in neutrophils may be found (27, 28).

**Acquired and congenital neutropenia**

An absolute neutrophil count (ANC) < 1500/μL is defined as neutropenia, which is graded on the severity of decreased ANC (Table I). Causes for neutropenia may be congenital or, more commonly, acquired. Neutropenia may be asymptomatic until an infection occurs. Benign neutropenia exists, and the individuals are not at risk for serious infection. However, onset of fever with neutropenia, termed febrile neutropenia, commonly occurs as a potentially life-threatening complication of chemotherapy and involves considerable cost as...
Neutropenia results from a growing list of germline mutations in genes, such as \textit{ELANE}, \textit{HAX1}, \textit{GFI1}, \textit{G6PC3}, \textit{WAS}, and \textit{CSF3R} (32). Soon after birth, children with SCN develop a grade 4 neutropenia. SCN is a lifetime condition resulting from increased apoptosis of granulocytic progenitors in the marrow. As a result of the severity and chronic nature of SCN, individuals are prone to recurrent infections, especially from the endogenous flora in the gut, mouth, and skin. Most cases of SCN are due to de novo mutations. Transmission may be autosomal dominant, recessive, or X-linked. The most common mutation involves \textit{ELANE} and is autosomal dominant (33, 34). Mutations in \textit{ELANE} encode the neutrophil elastase, a serine protease. \textit{ELANE} is expressed during granulopoiesis, maximally at the promyelocyte stage. It is hypothesized that mutations in \textit{ELANE} cause neutropenia via improper folding of the protein that triggers the unfolded protein response. Unfolded protein response–generated stress drives apoptosis due to an overload of unfolded protein, and an arrest in differentiation at the promyelocyte stage is observed. Fascinatingly, \textit{ELANE} mutations are also associated with cyclic neutropenia. Cyclic neutropenia is characterized by granulocyte nadirs $< 200/\mu l$ occurring every 21 d.

Patients with SCN are always at risk for life-threatening infections. Early phase 1 clinical trials held in 1989 (35, 36) evaluated G-CSF therapy for SCN and cyclic neutropenia. Both trials demonstrated a $\geq 10$-fold increase in neutrophil counts, reducing the severity of neutropenia from grade 4 to grade 1 to normal counts. A reduction in the days of cyclic neutropenia from 21 to 14 d was observed, and a consistent increase in ANC was observed in SCN. In 1990, two studies explored the benefit of G-CSF versus GM-CSF in treating congenital neutropenia. Gray collie dogs with cyclic neutropenia due to mutations in the endocytosis gene \textit{AP3B1} (37) were studied with three cytokines: G-CSF, GM-CSF, and IL-3. GM-CSF and G-CSF showed an expansion of neutrophil counts, but only G-CSF prevented the cycling of hematopoiesis (10). Similar to the dog study, G-CSF therapy increased ANC, whereas GM-CSF therapy increased eosinophil counts but not neutrophil counts (38). Following the beneficial effects of G-CSF in the above phase 1/2 studies, a phase 3 clinical trial was performed in 1993 (39). Patients with SCN, cyclic neutropenia, and idiopathic neutropenia ($n = 123$) were included in the study. Patients were randomly treated immediately or after a 4-mo observation period. Almost all of the patients (108/120) receiving G-CSF therapy displayed a restoration of ANC from grade 4 to normal levels. The increase in ANC resulted from increased production of neutrophils in bone marrow. Infection-related incidents were reduced by $\sim 50\%$ ($p < 0.05$), and antibiotic use was reduced by 70%.

One particular form of inherited neutropenia is WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome (40). Myelokathexis refers to a build-up of mature neutrophils in the bone marrow. Mutations in \textit{CXCR4} result in the syndrome (41). \textit{CXCR4} and its ligand SDF-1 mediate the retention of neutrophils. G-CSF administration leads to upregulation of SDF-1 and the subsequent release of neutrophils into the peripheral circulation (42). A recently published phase 1 study demonstrated the safety and efficacy of low-dose plerixafor, a CXCR4 antagonist (43). One widely used indication for G-CSF is to mobilize and harvest hematopoietic progenitor cells into the periphery for stem cell transplantation (44), and the concomitant use of plerixafor enhances the mobilization (45).

**Neutropenia associated with aplastic anemia**

Severe aplastic anemia (SAA) is a disease in which stem cells residing in the bone marrow are damaged, leading to a deficiency in all hematopoietic cell lines. SAA has a high mortality, but the $5$-year mortality is reduced to $<10\%$ with matched sibling stem cell transplantation or to $30\%$ with immunosuppressive therapy (IST) (46). IST includes antithymocyte globulin, cyclosporine, and glucocorticoids. The addition of G-CSF to IST was studied in a number of randomized studies. It was shown that G-CSF reduces the number of infectious complications and hospital days compared with standard therapy alone; however, its addition did not affect overall survival rates (47, 48). Although treatment with G-CSF or GM-CSF results in a neutrophil response, a sustained trilineage response was uncommon when used alone or in combination with other hematopoietic growth factors (49, 50). The response to G-CSF may have prognostic value. Patients treated with IST plus G-CSF who did not achieve a WBC count $\geq 5000/\mu l$ had a low probability of response and high mortality (51–53). Similarly, GM-CSF was studied as a potential adjunct to IST with similar results (48). These findings suggest that G-CSF and GM-CSF may be useful adjuncts to standard IST for SAA.

**Neutropenia associated with chemotherapy for solid tumors**

In 1991, the FDA approved the use of recombinant human G-CSF (filgrastim) to treat cancer patients undergoing myelotoxic chemotherapy. Multiple factors affect the severity of neutropenia, with the most important being the type and severity of chemotherapy dosage and the underlying disease (54, 55). In 1994, the American Society of Clinical Oncology (ASCO) recommended primary prophylaxis with G-CSF or GM-CSF for the expected incidence of neutropenia $\geq 40\%$ (56). The purpose of the guidelines was to reduce the incidence and length of neutropenia and, thus, the time of hospitalization, which would reduce costs significantly. Three prospective, randomized, placebo-controlled trials formed the basis of the recommendations. The first phase 3 trial tested the applicability of G-CSF as an adjunct to chemotherapy in patients treated for small cell lung cancer with cyclophosphamide, doxorubicin, and etoposide (57). A major outcome of the study was the significant reduction by at least one episode.

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<tr>
<th>Neutropenia Grade</th>
<th>Absolute Neutrophil Count</th>
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<tr>
<td>Grade 1</td>
<td>$\geq 1.5 \times 10^9/ml$</td>
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<td>Grade 2</td>
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<td>Grade 3</td>
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<td>Grade 4</td>
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of febrile neutropenia in 77% of those treated with G-CSF versus 40% in the placebo group. A reduction in the median duration of grade 4 neutropenia was observed in all cycles of chemotherapy (1 d in the G-CSF group versus 6 d in the placebo group). From a cost-benefit perspective, the data translated into a reduction in the 50% incidence of infection, antibiotic treatment, and days of hospitalization with G-CSF treatment versus placebo. A similar study performed in Europe in patients with small cell lung cancer also found that prophylactic G-CSF treatment reduced the incidence of febrile neutropenia (53% in placebo group versus 26% in G-CSF group) (58). A reduction in chemotherapy dose by 15% was indicated in 61% of the placebo group versus 29% of the G-CSF group. A gap ≥ 2 d in the chemotherapy treatment group was observed for 47% of patients in the placebo group and 29% of the patients in the G-CSF group. The third trial investigated G-CSF therapy in NHL treated with vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide, and bleomycin (59). The incidence of neutropenia was reduced for the G-CSF group (23%) versus the placebo group (44%), with fewer delays and shorter duration of treatment in the G-CSF-treated group. In comparison, GM-CSF trials provided less convincing data. In a trial for cyclophosphamide, vincristine, procarbazine, bleomycin, prednisolone, doxorubicin, and mesna administered as therapy for NHL, the use of molgramostim (GM-CSF) resulted in faster recovery from neutropenia and reduced hospitalization, but the benefit was limited to only 72% of the patients that could tolerate GM-CSF (60). Another trial with small cell lung cancer did not show any significant effect with molgramostim treatment (61).

The development of better chemotherapeutic regimens that were less myelotoxic provided more cost-effective options compared with CSF therapy. In many cases, the incidence of neutropenia was reduced to ≤10%. However, the advantage of CSF therapy in both increasing the intensity and maintenance of dose versus the cost of the growth factors was actively debated. In 2003, a large randomized study showed the benefit of G-CSF therapy with dose-dense chemotherapy (cyclophosphamide, paclitaxel, and doxorubicin) in patients with node-positive breast cancer (62). Significantly improved disease-free survival (risk ratio = 0.69, p = 0.013) were observed in patients receiving G-CSF. Fewer patients reported grade 4 neutropenia in the G-CSF group (6%) compared with the non-G-CSF group (33%). In 2004, two additional studies with older (60–75 y) and young (<60 y) NHL patients observed a reduction in chemotherapy regimens from 3 to 2 wk combined with an improvement in the rate of progressive disease and overall survival (63, 64). In 2005, two large trials supported the use of G-CSF in reducing the incidence of fever and neutropenia and suggested its use with the first cycle of chemotherapy (65, 66). The first study compared the effect of antibiotics versus antibiotics + G-CSF in small cell lung cancer patients undergoing cyclophosphamide, doxorubicin, and etoposide treatment. A significant reduction in the incidence of febrile neutropenia was observed for the antibiotics + G-CSF group (10%) compared with the antibiotics-only group (24%) (66). The second study investigated the effect of pegfilgrastim in breast cancer patients treated with docetaxel (65). Approved in 2001, pegfilgrastim was developed to improve the renal clearance rate (67), and a single dose provided similar or greater improvement in the ANC after chemotherapy compared with daily doses of filgrastim (68). The randomized, placebo-controlled trial conducted with 928 patients demonstrated a lower incidence of febrile neutropenia in patients receiving pegfilgrastim (1%) compared with placebo (17%). Hospitalization also was reduced in the pegfilgrastim group (1%) compared with the placebo group (14%). In 2005 and 2006, the National Comprehensive Cancer Network (http://www.nccn.org) and ASCO changed the risk threshold for contracting neutropenia from 40 to 20% to justify the use of myeloid growth factors as an adjuvant to chemotherapy in treating neutropenia (69). The use of myeloid growth factors, their cost effectiveness, and the duration of their use during chemotherapy remain of great interest to clinical oncologists. A randomized phase 3 study with a noninferiority design demonstrated the efficacy of G-CSF prophylaxis against febrile neutropenia in women with breast cancer for the entirety of their myelosuppressive treatment (70). Current guidelines from ASCO, the National Comprehensive Cancer Network, and the European Organization for Research and Treatment of Cancer recommend the use of myeloid growth factors when the risk for febrile neutropenia is ≥20% (71, 72).

Neutropenia associated with leukemia

Neutropenia in patients with leukemia results from both the underlying disease and aggressive chemotherapy. The ASCO guidelines developed in 1994, like for solid tumors, considered data obtained from three large randomized trials. Unlike the solid tumor trials, two of the three trials used GM-CSF versus G-CSF. The two GM-CSF trials reported conflicting findings, with some statistical significance in the recovery of ANC but no significant reduction in hospitalization or the incidence of serious infections (73, 74). The G-CSF trial showed a recovery in ANC, reduction in days of neutropenia, and a trend toward better recovery rates. However, like the GM-CSF trials, no improvement in days of hospitalization or usage of antibiotics was observed (75). Thus, a beneficial response by the growth factors was not observed in leukemia. However at the time of ASCO’s 2000 guidelines, newer placebo-controlled trials demonstrated a reduction in neutrophil recovery time from 6 to 2 d and reduced hospitalization times in the setting of induction chemotherapy (76). The 2000 ASCO guidelines also identified a potential benefit for growth factor therapy in consolidation chemotherapy. The 2006 update did not introduce any significant changes and recommended the application of CSF therapy postinduction and consolidation therapy (69).

Unlike chemotherapy-induced neutropenia, congenital neutropenia patients experience neutropenia for life and require long-term treatment with G-CSF. The long-term effects of G-CSF therapy have become important in the management of congenital neutropenia. Patients receiving G-CSF therapy for as long as 8 y were evaluated for safety and efficacy (77). Neutrophil counts were maintained without exhaustion of myelopoiesis. A significant improvement in the quality of life was achieved by the reduction in antibiotic treatment and hospitalization time, allowing for normal growth, development, and participation in normal daily activities. The SCN international registry was formed in 1994 to further assess the progress of SCN patients being treated with G-CSF. A 10-y report that followed patients with SCN (n = 526) who were being treated with G-CSF was released in 2006 (78). Consistent with pre-
vious reports, an increase in ANC was observed in majority of the patients with an overall improvement in quality of life.

Leukemia transformation is significantly higher in SCN patients, and the SCN international registry reported that 21% of patients with SCN developed leukemia while being treated with G-CSF. Although leukemic transformation has been reported in SCN patients before the development of G-CSF therapy (79), the precise role of G-CSF therapy in leukemic transformation remains unknown. Almost all SCN patients undergo G-CSF therapy; thus, it is difficult to assess leukemic transformation in the absence of G-CSF treatment. However, patients who require higher doses of G-CSF are at a higher risk for developing MDS/AML (80).

Germline mutations in CSF3R, which encodes G-CSFR, are infrequent causes of SCN and result in refractoriness to filgrastim (81). Acquired nonsense mutations in CSF3R were observed in ~80% of SCN patients who progressed to secondary MDS/AML (82–86). The nonsense mutations result in deletion of the C terminus of G-CSFR, resulting in the loss of one to all four tyrosine residues and the inability to undergo normal ligand-induced internalization and endosomal routing (87, 88). The truncated receptor mutants produce a phenotype of enhanced proliferation and impaired differentiation in response to G-CSF. Furthermore, knock-in mice harboring a similar mutation showed hyperproliferative responses to G-CSF administration and strongly prolonged activation of STAT5, which are implicated in increased hematopoietic progenitor stem cell expansion in vivo (89). This prediction was validated in a patient with SCN who developed secondary AML concomitant with a nonsense mutation of G-CSFR. Upon discontinuation of G-CSF and without chemotherapy, the blast count in the blood and bone marrow disappeared, although the mutation remained detectable (90). The tight correlation between the acquisition of G-CSFR mutations and progression of SCN to secondary MDS/AML and the abnormal signaling features in vitro and in vivo strongly suggested that mutated CSF3R could be a driver of myelodysplasia. Data from recent studies, which showed that the CSF3R T595I mutation is the most prevalent mutation found in chronic neutrophilic leukemia and that treatment with the Jak2 inhibitor ruxolitinib resulted in marked clinical improvement, support the hypothesis that mutations in G-CSFR are indeed drivers of myeloproliferative disease (91, 92). A low frequency of CSF3R mutations also occurs in AML and chronic myelomonocytic leukemia (93).

Future developments

More than 20 y after its registration by the FDA, biosimilars (94) of G-CSF are being developed (95). (Amgen lost its patent protection for filgrastim in 2008, after developing worldwide sales ~$4.5 billion.) The European Medicine Agency approved six biosimilars to G-CSF, and in February 2015, the FDA approved the first biosimilar, filgrastim-sndz, in the United States since passage of the Biologics Price Competition and Innovation Act. Teva’s tbo-filgrastim was approved in the United States in 2012 before that legislation. These drugs must demonstrate “high similarity” in the molecular characterization, purity, stability, pharmacokinetics, pharmacodynamics, clinical efficacy, tolerability, and safety to the original agent (69). Pricing analysis suggests that that use of biosimilars to filgrastim, bevacizumab, trastuzumab, and rituximab may save up to $44.2 billion over the next 10 y (96).

G-CSF and/or GM-CSF may improve chemotherapy and immunotherapy of hematologic malignancies and nonblood cancers. For instance, these myeloid growth factors can recruit quiescent leukemic cells into the cell cycle for enhanced killing from cell cycle–specific chemotherapy (74, 97). As a proinflammatory cytokine, GM-CSF is being used to promote dendritic cell activity in a variety of anticancer trials. Indeed, GM-CSF is approved as part of the sipuleucel-T regimen for the treatment of hormone-resistant prostate cancer. There, dendritic cells are incubated with a fusion protein consisting of prostatic acid phosphatase and GM-CSF. Although sipuleucel-T has been underused, in part because of its expense, GM-CSF is being studied in the context of other immunotherapeutic interventions (https://clinicaltrials.gov).

G-CSF has immunomodulatory effects on immune cells. G-CSF enhances Ab-dependent cellular cytotoxicity and cytokine production in neutrophils (98). However, it also inhibits TLR-induced proinflammatory cytokines produced by monocytes and macrophages (99). CD34+ monocytes that inhibit graft-versus-host disease are mobilized in response to G-CSF (100). In addition, G-CSF inhibits LPS-induced IL-12 production from bone marrow–derived dendritic cells cultured in vitro (101). Interestingly, administration of GM-CSF has the opposite effect, inducing cytokine production in the circulation in response to LPS (102).

GM-CSF pathways may be high-value targets in autoimmune diseases. For example, inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract caused by a combination of environmental and genetic factors. Crohn’s disease and ulcerative colitis can be difficult to treat, and relapse of disease can occur at any time. Biochemical markers identifying patients at risk for relapse are currently lacking. GM-CSF signaling was recently implicated in the pathogenesis of Crohn’s disease. GM-CSF is required for myeloid cell antimicrobial functions and homeostatic responses to tissue injury in the intestine (103). Preliminary studies found that GM-CSF reduces chemically induced gut injury in mice (104). In human studies, higher concentrations of circulating Abs against GM-CSF are found in patients with active IBD compared with those with inactive disease (103). Currently, several studies and clinical trials are looking at the use of GM-CSF in the treatment of IBD and anti–GM-CSF Ab for the monitoring of disease activity and assessing the risk for recurrence (105, 106).

Pulmonary alveolar proteinosis (PAP) is a rare disorder characterized by accumulation of periodic acid–Schiff+ lipoproteinaceous material in the alveoli of the lung, leading to impaired gas exchange, respiratory insufficiency, and, in severe cases, respiratory failure (107). Autoimmune PAP accounts for 90% of cases and is due to the presence of autoantibodies against GM-CSF. Hereditary PAP is caused by mutations in the genes CSF2RA and CSF2RB that code for the α and β subunit of GM-CSFR, respectively (108, 109). In autoimmune PAP, the presence of anti–GM-CSF Abs leads to aberrant in vivo GM-CSF signaling that is required for the macrophage-mediated clearance, but not uptake, of pulmonary surfactant. This results in the progressive accumulation of foamy surfactant-laden macrophages and intra-alveolar surfactant in the alveoli of the lung (110). The gold standard of therapy has been whole-lung lavage. Although an effective therapy, it often needs to be repeated as a result of reaccumulation of lipoproteinaceous
sediment and is not without complications. Newer therapies have been studied, including pulmonary macrophage transplanation, plasmapheresis to remove the GM-CSF autoantibody, and inhaled GM-CSF (111–113). Inhaled GM-CSF is of particular interest because it was shown to be safe and effective in animal studies and phase 1 and 2 clinical trials (114, 115).

Disclosure

The authors have no financial conflicts of interest.

References


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