

Review

A Role for G-CSF (Granulocyte-Colony Stimulating Factor) in the Central Nervous System

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KEY WORDS

G-CSF, stroke, cerebral ischemia, apoptosis, hematopoietic growth factor, neurogenesis, regeneration

ABBREVIATIONS

BBB	blood-brain barrier
BrdU	bromodesoxyuridine
CNS	central nervous system
EPO	erythropoietin
ERK	extracellular-signal-regulated kinase
G-CSF	granulocyte-colony stimulating factor
MCAO	middle cerebral artery occlusion
PI3-K	phosphoinositol-3 kinase
SGZ	subgranular zone
STAT	signal transducer and activator of transcription
SVZ	subventricular zone

ABSTRACT

G-CSF (granulocyte-colony stimulating factor) is a hematopoietic growth factor that has been known for 20 years, and has been named for its role in the proliferation and differentiation of cells of the myeloid lineage. We have uncovered a novel spectrum of activities of G-CSF in the central nervous system. G-CSF and its receptor are expressed by neurons in many brain regions, and are upregulated upon experimental stroke. In neurons, G-CSF acts anti-apoptotically by activating several protective pathways. In vivo, G-CSF decreases infarct volumes in acute stroke models in rodents. Moreover, G-CSF stimulates neuronal differentiation of adult neural stem cells in the brain, and improves long-term recovery in more chronic stroke models. Thus, G-CSF is a novel neurotrophic factor, and a highly attractive candidate for the treatment of neurodegenerative conditions. Here we discuss this new property of G-CSF in contrast to its known functions in the hematopoietic system, summarize data from other groups on G-CSF's actions in cerebral ischemia, compare G-CSF to Erythropoietin (EPO) in the CNS, and highlight clinical implications.

BACKGROUND: KNOWN CHARACTERISTICS OF G-CSF

Granulocyte colony-stimulating factor (G-CSF) is a 19.6 kDa glycoprotein that was identified initially as a serum activity that induced differentiation of the murine myelomonocytic leukemic cell line WEHI-3B.^{1,2} It was cloned 20 years ago and found to be a 207 amino acid protein, with a hydrophobic signal sequence of 30 amino acids.³ Differential splicing of the G-CSF mRNA can lead to the production of two variant forms of this protein with one of the resulting proteins shortened by three amino acids. The ability to produce G-CSF is characteristic of many cell types after appropriate stimulation. Monocytes are the most prominent source of it, but also mesothelial cells, fibroblasts and endothelial cells have been found to produce it.⁴⁻⁷ In addition, a variety of tumours have also been reported to produce G-CSF.^{8,9} Production of G-CSF can be induced in vitro by TNF- α , IL-1, GM-CSF, IL-4 and bacterial LPS.^{4,5,7,10,11}

Initial characterization of the human G-CSF receptor was reported by Nicola et al., and a further description of the biochemical and molecular nature of the G-CSF receptor was provided later.¹²⁻¹⁶ The G-CSF receptor (CD114; G-CSFR) is a typical cytokine receptor with one transmembrane domain, and an intracellular signal transduction domain, and homo-oligomerizes upon ligand binding. Receptors for G-CSF are present on precursors and mature neutrophilic granulocytes (300–1000 receptors on each), monocytes and platelets, but have not been found on erythroid, eosinophilic or lymphoid cells.¹⁷⁻¹⁹ In addition, G-CSF receptors have been found on the surfaces of nonhematopoietic cells, including endothelial cells and small-cell lung cancer cells.^{20,21}

Soon after cloning of G-CSF its clinical potential was realized to counteract chemotherapy-induced neutropenia.²²⁻²⁴ Today, G-CSF has been given to over three million patients worldwide for this and related indications such as bone-marrow harvesting, idiopathic neutropenias, and appears as a well-tolerated drug.

G-CSF'S FUNCTION IN THE BRAIN

We have recently discovered that G-CSF plays a prominent role in another body compartment, the central nervous system, and is of potential relevance to a number of neurological conditions.²⁵ Initially, we and others discovered that G-CSF decreased infarct volume in rodent stroke models.^{26,27} A first indication of a potential direct effect on cells of the brain came from the observation that G-CSF had a direct protective effect in cultured

neurons against glutamate-induced cell death.²⁶ We have demonstrated that the hematopoietic factor G-CSF is in fact an endogenous protein expressed in neurons that is upregulated upon ischemia in regions at risk ("penumbra"), and provides protection against programmed cell death in neurons, reflected by a robust neuroprotective activity in acute stroke models *in vivo*.

Another fully unexpected activity of G-CSF was discovered by the finding that adult neural stem cells (NSCs) in the brain expressed its receptor. NSCs reside in several regions in the adult brain, and give rise to mature neuronal cells. These cells can be cultivated, and used for differentiation assays. G-CSF induced a neuronal differentiation phenotype in these cells *in vitro*, an effect that correlated to *in vivo* induction of neurogenesis and subsequently enhanced functional recovery after cortical cerebral ischemia.²⁵

EVIDENCE FROM OTHER GROUPS

In the last two years, the beneficial actions of G-CSF in various stroke models have been demonstrated by a number of independent researchers throughout the world.²⁵⁻³⁰ The outcome measures used and the conclusions drawn are, however, quite different, and we will discuss these studies in light of G-CSF's mechanisms-of-action in the CNS outlined above. Six et al. have used the transient middle cerebral artery occlusion model in mice, and reported infarct size reduction and drastic improvement of mortality after 4 days when treatment with G-CSF was initiated 24 h after onset of ischemia.²⁷ The authors speculated about an indirect mechanism of action: G-CSF could have induced mobilization of bone-marrow derived stem cells that invade the infarcted brain, and improve outcome after stroke. This capacity of bone-marrow derived cells has indeed been demonstrated (reviewed in ref. 31), but the mechanism of their advantageous action remains unclear. The proposed transdifferentiation of bone marrow derived cells into neural cells that could induce functional and structural recovery poststroke was recently doubted by a number of labs (e.g., refs. 32 and 33). This mechanism was also propagated by Shyu et al.²⁸ Using combined CCA/distal MCA occlusion, these authors assessed neurological behavior up to 28 days after ischemia. Treatment of 5 x 50 µg/kg bodyweight/day initiated 24 hours after stroke onset induced a significant behavioral improvement, and reduced infarct volumes. The authors used BrdU-labeling for identification of newborn cells, and detected more labeled cells in the ipsilateral hemisphere after G-CSF treatment, although quantitative data remain unclear in this paper. The authors concluded that such marked cells originated from bone-marrow derived stem cells, although specific markers for identification of blood derived cells were not presented. Indeed, most labeled cells were seen in the sub-ventricular zone, often colabeled with neuronal markers, arguing strongly that these cells originated from adult neural stem cells in concordance with our results. Interestingly, the authors also found enhanced labeling in blood vessel walls, suggesting a role of G-CSF in arteriogenesis following stroke.

Gibson et al. treated mice subjected to MCAO with a single dose of G-CSF 1 h after onset of ischemia.²⁹ They reported both an infarct reducing effect 2 days after stroke, and a long-term functional and cognitive improvement measured by the Morris water maze test. This suggests that beyond the sensorimotor improvement, which we

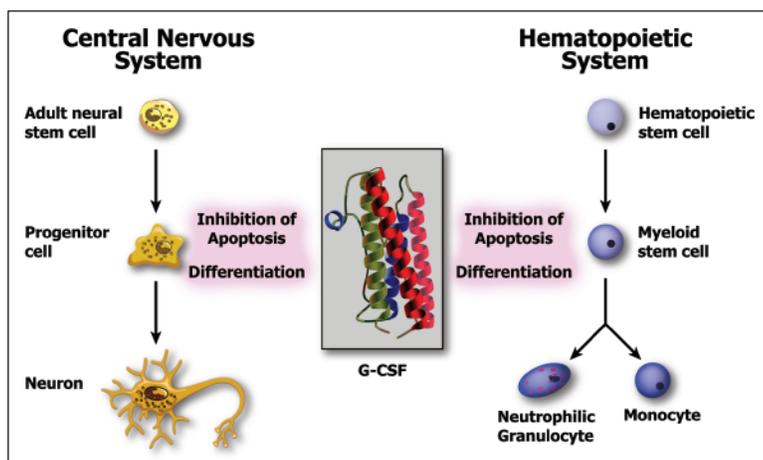


Figure 1. G-CSF has a previously unrecognized function in the central nervous system, that parallels its role in the hematopoietic system in its basic cellular activities. In the hematopoietic system, G-CSF drives the generation of neutrophilic granulocytes from myeloid precursor cells by its basic anti-apoptotic and pro-differentiation actions (right). In cells in the nervous system, G-CSF stimulates the differentiation of neurons from adult neural stem cells, and protects these cells by counteracting apoptosis (left). Thus, although active in two different body compartments, G-CSF's basic cellular functions appear remarkably preserved.

have determined, an additional pro-cognitive effect of G-CSF might contribute to long-term recovery after stroke. This effect on spatial memory and learning may likely also be associated with the increase in hippocampal neurogenesis we have observed. The most recent addition to the range of papers on G-CSF in stroke models is from Komine-Kobayashi and colleagues.³⁰ Following the above mentioned hypothesis, which holds bone-marrow derived stem cells responsible for G-CSF's positive action in stroke, they subjected chimeric mice with EGFP-expressing bone-marrow derived cells to cerebral ischemia. The authors report that migration of bone-marrow derived monocytes was not increased at all after G-CSF treatment, but rather decreased.

In conclusion, the broad array of data from different laboratories underline our results of a very stable neuroprotective and pro-regenerative effect of G-CSF in various stroke models, and are perfectly compatible with the two main direct mechanisms of G-CSF-mediated effects in the brain uncovered by us: anti-apoptosis and neurogenesis. Parenthetically, the published data point towards an astonishing time window for G-CSF in proximal or distal MCAO models: Two groups^{27,28} report effects on direct infarct parameters when treatment was initiated as late as 24 h after stroke onset.

PARALLELS TO G-CSF'S ROLE IN THE HEMATOPOIETIC SYSTEM

At the myeloid progenitor-cell level, G-CSF stimulates the growth of neutrophil granulocyte precursors.¹ In mature, i.e., postmitotic, neutrophils, G-CSF regulates survival³⁴ by inhibition of apoptosis.³⁵ We found evidence for the conservation of intracellular pathways from hematopoietic cells to neurons, for example, G-CSF elicits activation of proteins of the Stat family, or the PI3-K/Akt pathway. The basic cellular functions of G-CSF appear conserved in the nervous system, i.e., inhibition of apoptosis, and stimulation of cell differentiation (see Fig. 1). However, this is not the first example of a hematopoietic cytokine active in the blood system that also has a role in nerve cells. The best-studied example other than G-CSF is

erythropoietin (EPO, see detailed comparison below). The question why such cytokines specialized for one or a few cell lineages have also acquired a function in the nervous system is of course difficult to answer. It is however interesting to note that a high similarity between neural and hematopoietic stem cells has been detected by gene expression profiling.³⁶ The JAK/Stat pathway is present in most cells, and the more than 20 cytokine receptors in mammals that utilize this pathway have probably evolved from a common ancestor.³⁷ It is conceivable that during functional and cell-type-specific divergence of the different ligand/receptor pairs several have retained functions in phenotypically differing cell types and body compartments.

PARALLELS AND DIFFERENCES TO EPO

G-CSF is the second hematopoietic growth factor after Erythropoietin (EPO) which has a broad role in the central nervous system, that is likely as important as its function in the blood-forming system. Indeed, G-CSF parallels EPO in many respects. The idea that EPO has a role in the CNS dates back to 1993 when the EPO receptor was first described on the neural cell line PC12,³⁸ and when it was identified in the brain 2 years later.³⁹ While both the EPO and G-CSF receptor, and their respective ligands appear to be expressed by neurons in many brain areas, only the EPO receptor and ligand appear expressed in astrocytes.⁴⁰⁻⁴² Costainings of the G-CSF and EPO receptor and their respective ligands should light up how the expression patterns overlap or differ in the brain, and whether this might allow conclusions as to possibly differing functions in the brain.

Thereafter, many studies have proven a neuroprotective potential of EPO in cerebral ischemia.⁴³⁻⁴⁵ Furthermore, EPO (like G-CSF) crosses the blood-brain barrier.^{45,46,47} The EPO (like the G-CSF) receptor is a cytokine-type one-transmembrane protein, however, there are indications that the EPO receptor in the brain responsible for the neuroprotective action is a heterodimer of the EPO-receptor and the common β -chain receptor (that forms the common part of the receptors for the cytokines IL5, IL3 and GM-CSF).⁴⁸ Concerning the neuroprotective actions of G-CSF, we have no evidence so far to assume that the CNS-receptor for G-CSF might have a composition different from the known homo-oligomer.

Concerning the signal transduction events that ultimately lead to cellular protection there are also strong similarities, because the EPO receptor also lacks an intrinsic tyrosine kinase activity, and recruits the Janus kinase 2 for signaling. We have shown that G-CSF activates ERK1/2 and 5, Stat3 and PI3K-Akt signaling, and demonstrated that at least Akt activation is crucial for G-CSF's anti-apoptotic actions on neurons. EPO-induced PI3K-Akt signaling has also been shown to be a major player in EPO-mediated neuroprotection,⁴⁹⁻⁵² although this may not be the case in all experimental paradigms.⁵³ Also, ERK1/2 kinases have been implicated in EPO-related neuroprotection.^{50,53} At the moment data are insufficient to decide how the pathways evoked by G-CSF and EPO differ. It will be highly interesting to examine these pathways in parallel experiments, and see whether there is any superadditive effect of G-CSF and EPO treatment.

Apart from the anti-apoptotic action of G-CSF on neurons, we discovered a strong impact of G-CSF on neurogenesis in the adult animal. EPO also harbors a neurogenic potential,^{54,55} however, only a experiments designed in parallel would allow to judge potential similarities and differences.

CLINICAL IMPLICATIONS

Hematopoietic factor signaling appears as a novel protective system in the brain that counteracts key mechanisms in acute stroke pathology, and enhances stroke recovery, mediated at least in part via the formation of new neurons. For therapeutic purposes, factors such as EPO or G-CSF ideally fulfill the criteria of a novel type of stroke drug, and activate dual mechanisms of action in acute and chronic stroke pathology. For G-CSF, systemic immunomodulatory effects⁵⁶ might be an additional benefit in stroke patients, whereas in the case of EPO variants are now available that circumvent undesired systemic effects.^{57,58} We have initiated a randomized, multicenter, placebo-controlled phase IIa trial with G-CSF ("AXIS")⁵⁹ to establish tolerability of this protein in the acute stroke situation. Furthermore, our data suggest that G-CSF may enhance brain function even long after a stroke, or in healthy subjects.²⁵ Therefore, G-CSF might also be suitable as supportive treatment in rehabilitation phases after stroke. Moreover, although no animal studies have been published to date, it appears plausible from the mechanisms-of-action that G-CSF might also have beneficial activity for chronic neurodegenerative conditions such as Parkinson's disease, or amyotrophic lateral sclerosis (ALS).

Besides all arguments in favor of G-CSF as a novel type of neuroprotective drug, particularly due to its multiple mechanism-of-action, and broad preclinical proof-of-principle, one major advantage of this protein is that we are dealing with a drug with a well-known pharmacological profile, and an excellent safety record. This is an invaluable advantage in a situation where unexpected side effects, or the fear thereof, have stopped a number of promising drugs in the field of stroke and neurodegeneration.

PERSPECTIVE

We have identified G-CSF as a novel neurotrophic factor, and ascertained a role in neuroprotection and neurogenesis relevant to ischemia. A lot of exciting questions remain open: Does G-CSF have a role during development of the nervous system? Are there any additional properties of G-CSF for neurons typical of other neurotrophic factors (e.g., neurite outgrowth promoting activity)? Which signal transduction pathways are relevant to G-CSF's neurogenic action? Is G-CSF effective in other neurodegenerative disease models besides cerebral ischemia? The next years will undoubtedly see a large array of publications that address these and other questions.

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